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ABSTRACT

This teacher's guide provides information and resources for helping to familiarize students with chemistry and its everyday applications around the world using inquiry and investigations. Contents include: (1) "Introducing Molecules in Living Systems"; (2) "Considering Life Processes"; (3) "Understanding the Structure of Biomolecules"; (4) "Properties and Reactions of Biomolecules"; (5) "Enzymes: Where the Action Is?"; (6) "Metabolism: The Community of Enzyme Reactions"; (7) "The Organization of Cellular Activities"; and (8) "Where Are We?" (YDS)

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interdisciplinary approaches to chemistry

IAC

TEACHER'S GUIDE

MOLECULES IN LIVING SYSTEMS

A BIOCHEMISTRY MODULE

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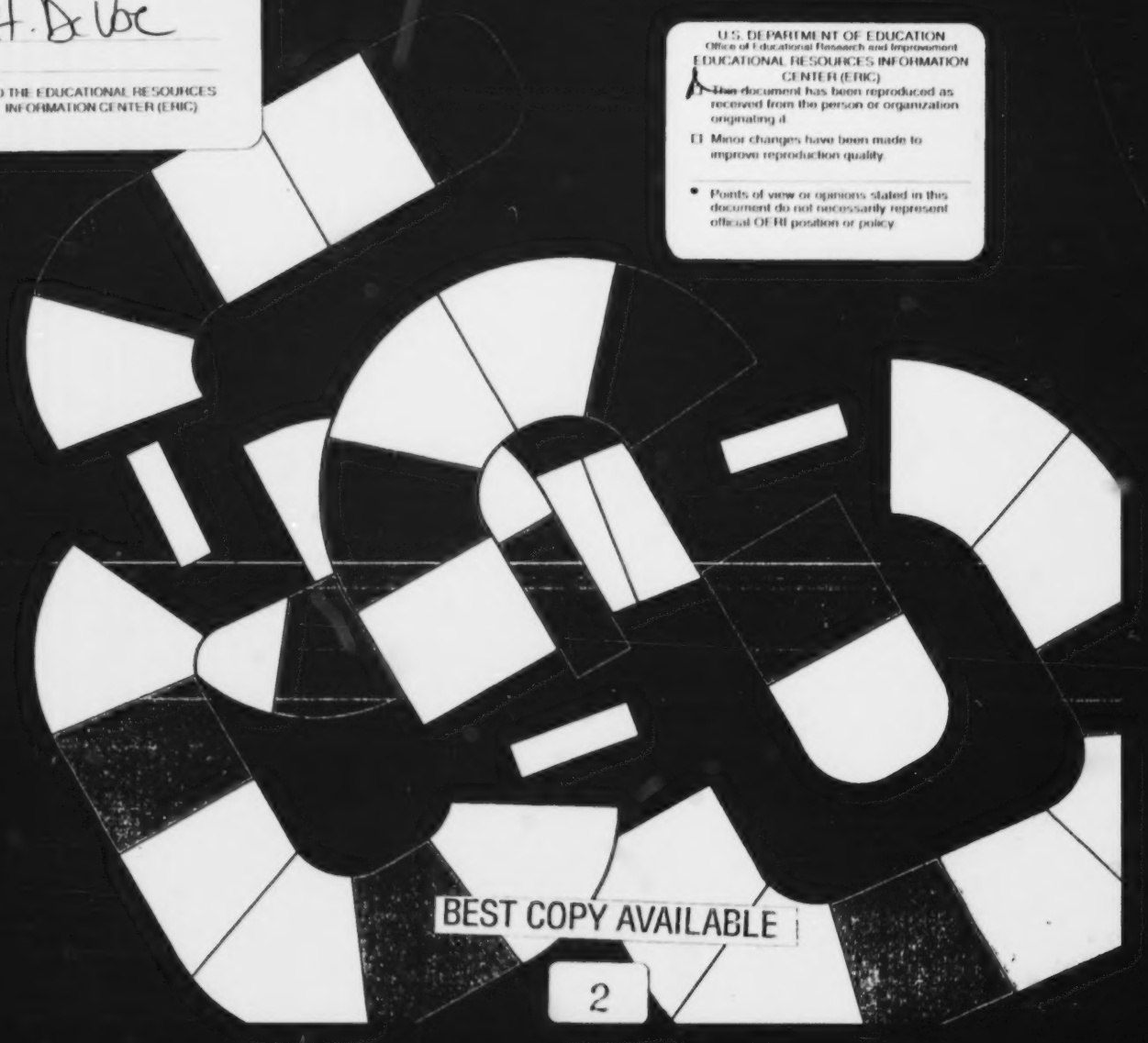

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TEACHER'S GUIDE

MOLECULES IN LIVING SYSTEMS

A BIOCHEMISTRY MODULE

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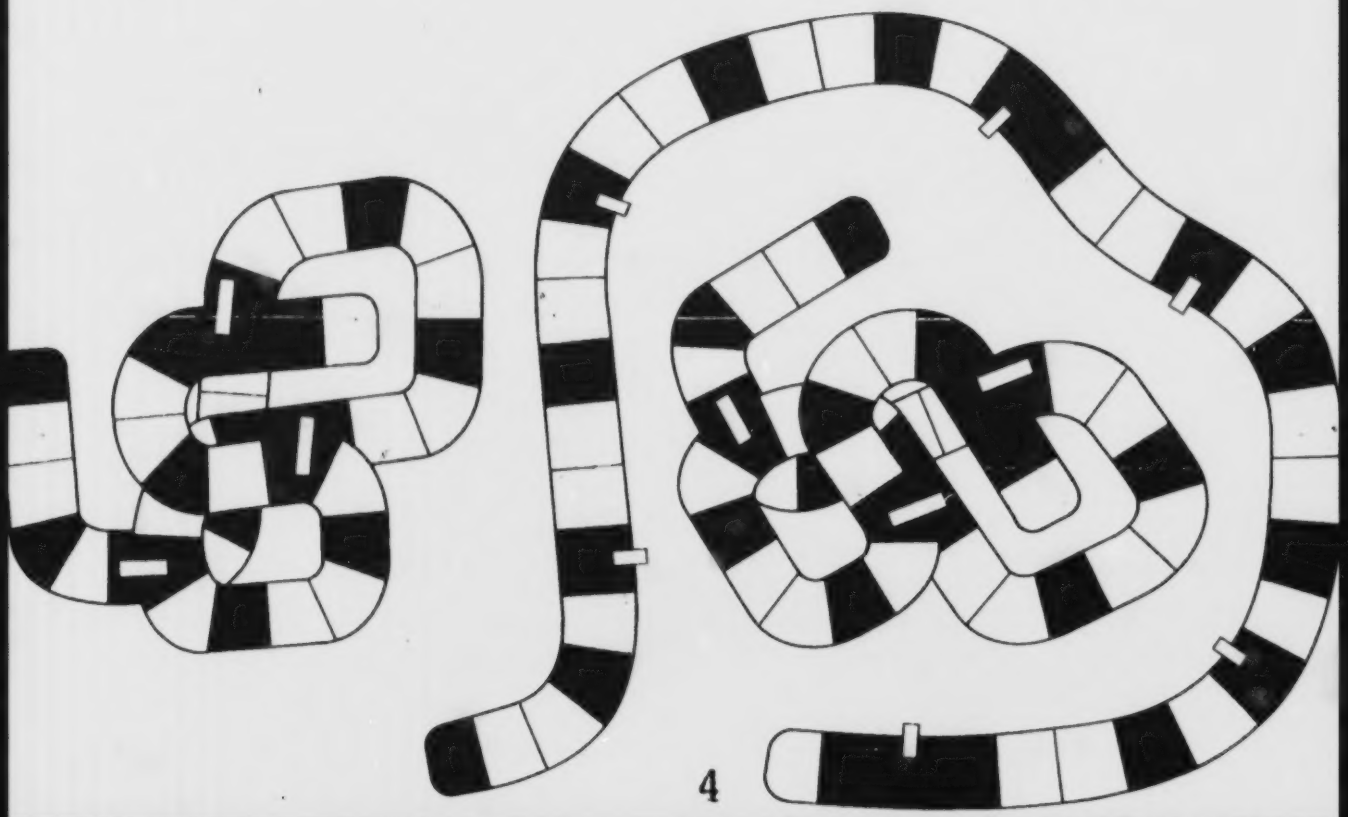

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TEACHER'S GUIDE

MOLECULES IN LIVING SYSTEMS:
A BIOCHEMISTRY MODULE

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Introducing Molecules in Living Systems

Molecules in Living Systems: A Biochemistry Module was developed to give high-school students a means of comprehending biochemistry in a practical way. The introductory section, entitled *The Chemistry of Life*, sets the theme—a molecular view of life—which is carried through to the summary, "Where Are We?"

As with other modules in the IAC program, the text of *Molecules in Living Systems* is easy to read; its approach is both light and serious. This module also is laboratory oriented, allowing students the opportunity to work directly in exploring roles of biomolecules in chemical reactions. Students will investigate enzymes and catalysts, test for protein denaturation, study the process of fermentation by making sauerkraut, and use simple materials to make an artificial cell membrane in order to understand the complexity of the cell. Students will be able to appreciate the complexity of biochemistry and how it plays a vital part in each of our lives.

We have introduced new topics and discussions in this revised edition. Many areas are considered in great detail, while other areas that still challenge the research biochemist are discussed more generally, leaving it open to the inventiveness and creativeness of both teacher and student to explore new topics with this module as the starting point. We do not suggest limiting the use of this module to chemistry courses only. *Molecules in Living Systems* can be used in conjunction with biology courses that cover molecular aspects of living systems as well as nursing or health-related courses. This is one of the unique features of the IAC series; it is interdisciplinary.

The material is current and in many instances moves the student to the frontiers of biochemistry research. It is not our intent that this course develop practicing biochemists, but that students have a broader understanding of the problems, issues, and decisions that face them every day. We would also like students to share a feeling of respect for and pride in the many people who have spent their lives studying this fascinating subject.

Special Features in the Student Module

Metric System Le Système Internationale (SI) is used throughout the IAC program. As you work with this module, you may wish to review some points of the metric system as presented in *Reactions and Reason: An Introductory Chemistry Module* (see section *A-8* and Appendix II). There is a metric-units chart in the appendix of the student module that students can easily refer to.

Time Machine A feature we call the *Time Machine* appears in the IAC modules in order to show chemistry in a broader context. For some students, this may provide a handle on particular aspects of chemistry by establishing the social-cultural-political framework in which significant progress was made in chemistry. Students may enjoy suggesting other events in chemistry around which to create *Time Machines* of their own.

Cartoons A popular feature of the IAC program is the use of chemistry cartoons. These cartoons give students a chance to remember specific points of chemistry in another important way—by humor. Suggest that your students create other chemistry cartoons for their classmates to enjoy.

Safety Laboratory safety is a special concern in any chemistry course. In addition to including safety discussion and guidelines in the appendix of each student module and each teacher's guide, experiments have been developed in a way designed to eliminate potentially dangerous chemicals or procedures. Moreover, each experiment that might present a hazard—through fumes, corrosive chemicals, use of a flame, or other con-

ditions—has been marked with a safety symbol to alert students and teachers to use added, reasonable caution. Caution statements, in bold type, also appear in experiments to specifically instruct the student on the care required.

Selected Readings Articles and books that tie in with the topics discussed in the IAC program have been listed in the appendix of the student module as well as in the teacher's guide. Encourage your students to use this section. You may wish to suggest other material that you yourself have found interesting and enjoyable.

Illustrations and Photographs The module is extensively illustrated to provide relevant and stimulating visual material to enable students to relate chemistry to everyday life, as well as to provide material for provocative discussion. In using some of these illustrations, it is not the intention of IAC to endorse any particular product or brand, but only to relate chemistry to life outside the classroom. As you proceed through each section, encourage students to collect, display, and discuss photos and illustrations that provoke further discussion.

Questions A number of questions, problems, and exercises have been interspersed throughout the student module, in addition to the questions that are naturally built into the narratives and the laboratory experiments. You will find some of these problems in specifically marked sections in the student module. These questions can be used in a variety of ways as you see fit. They are not planned as tests—remember, the IAC program is designed so that mastery of the content and skills can be achieved through the repeated reinforcement of ideas and procedures encountered by students as they progress through the various modules.

Managing the Laboratory

In the teacher's guide, hints and suggestions are given for managing each experiment in the laboratory. Share as many of these hints as possible with your students. Allow them to participate fully in successful laboratory managment. Make sure that you rotate assignments so that all students get a chance to experience this type of participation.

Preparations and Supplies Student aides can be helpful in preparing solutions, labeling and filling bottles, cleaning glassware, and testing experiments. (You should still test each experiment in the module to determine if any revision is needed to meet the needs of your students.)

Cleaning Up Involve your students in putting away equipment, washing up glassware, and storing material for the next time it is to be used. Taking care of equipment is part of the responsibilities we seek to foster in the students' outside environment.

Laboratory Reports You may have your own methods of student reporting. We are including some of the suggestions that IAC teachers have found successful in the past. It is helpful for students to keep a laboratory notebook. A quadrille-ruled laboratory notebook with a sheet of carbon paper allows a student to produce two data sheets and report summary copies. One copy of each page can be permanently retained in the notebook, while the duplicate copy can be submitted for evaluation or tabulation.

A realistic view of laboratory work suggests that, in the most fundamental sense, there are no "wrong" laboratory results. All students obtain results consistent with particular experimental conditions (either correct or incorrect) that they establish. Careful work will yield more precise results, of course. Encourage each student to take personal pride in experimental work. If students disagree on a result, discuss the factors that might account for the difference. A student who provides a thoughtful analysis of why a particular result turned out to be "different" (for example, incomplete drying, a portion of the original sample was spilled) deserves credit for such interpretation.

Laboratory Safety To use the IAC program safely, you should become thoroughly familiar

with all student activities in the laboratory. Do all the experiments and carry out all the demonstrations yourself before presenting them to your class. We have tested each experiment and have suggested the use of chemicals that present the least chance of a problem in laboratory safety. This teacher's guide has many suggestions for helping you provide your students with safe laboratory experiences. Have the students read the safety section in the appendix of the student module. Conduct a brief review of laboratory safety before allowing them to encounter their first laboratory experience in this module. Review safety procedures when necessary and discuss caution and safety each time a safety symbol appears in the student text.

Materials for IAC In light of increasing costs for equipment and supplies, as well as decreasing school budgets, we have tried to produce a materials list that reflects only the quantities needed to do the experiments, with minimal surplus. Thus, the laboratory preparation sections contain instructions for only a 10–20 percent surplus of reagents. Add enough materials for student repeats and preparation errors.

Evaluating Student Performance

There are many ways of evaluating your students' performances. One of the most important forms of evaluation is observing your students as they proceed through the IAC program. IAC has developed skill tests and knowledge tests for use with this module. These test items have been suggested and tested by IAC classroom chemistry teachers. You are encouraged to add these to your own means of student evaluation.

In addition to the problems and questions incorporated in the student module text and illustration captions, there are suggested evaluation items at the end of each module section in the teacher's guide. The module tests are at the end of the teacher's guide. Answers to all of the evaluation items are included to help you in your classroom discussion and evaluation.

Module Concepts

CONSIDERING LIFE PROCESSES

- Biochemistry is the study of molecules that play a role in living systems.
- There are similarities in the activities of living things.
- Biomolecules are classified by their biochemical function as well as their chemical structures.
- Ordinary chemical laws can be applied to biomolecules.

UNDERSTANDING THE STRUCTURE OF BIOMOLECULES

- Lewis dot structures provide a model for molecular structure.
- The covalent bond is a result of sharing electrons.
- Energy is stored in the body in the form of lipids and carbohydrates.
- Amino acids are biomolecules containing an amino group and a carboxylic acid group.
- Proteins are biomolecules composed of amino acids joined by peptide bonds.

PROPERTIES AND REACTIONS OF BIOMOLECULES

- Lipids can be distinguished from other biomolecules by their solubility in nonpolar solvents.
- The typical reactions of functional groups can be used to distinguish classes of biomolecules.
- An acid is a proton donor, and a base is a proton acceptor.
- A zwitterion is a compound that contains both positively charged functional groups and negatively charged functional groups.

ENZYMES: WHERE THE ACTION IS

- Enzymes are proteins that act as catalysts in biochemical reactions.
- The catalytic properties of enzymes are related to their structures.
- The structure of a protein depends upon the sequence of the constituent amino acids.
- Every protein has a unique, folded structure.
- The folds in a protein are maintained by four types of bonds or forces.
- The forces and bonds involved in the folding of proteins are:
  a. hydrogen bonds
  b. ionic bonds
  c. disulfide bridges
  d. hydrophobic bonds
- Enzyme reactions are influenced by factors such as temperature, pH, and active site availability.
- Protein chains can be unfolded (denatured) by heat and extremes in pH.
- The pH of a solution is a quantitative measure of the concentration of hydrogen ions in that solution.
- The specificity of an enzyme is a result of the nature of its active site.

METABOLISM: THE COMMUNITY OF ENZYME REACTIONS

- Metabolism refers to all the catalyzed reactions that take place in an organism.
- Digestion can be viewed as the first step of metabolism.
- The reactions in digestion are all of one basic type—namely, hydrolysis.
- Most complex biomolecules must be hydrolyzed to be absorbed through the intestine.
- Metabolism can be subdivided into metabolic pathways.
- Metabolic pathways contain enzymes, metabolites, and cofactors.
- Cofactors are substances that are required by enzymes to carry out their reactions.
- Some cofactors contain vitamins as a part of their structure.
- The metabolism of glucose can be broken down into three pathways: glycolysis, the Krebs cycle, and the respiratory chain.
- The complete metabolism of glucose results in the formation of ATP.
- ATP is the major biomolecule that provides the energy needed for the energy-requiring reactions.
- In general, metabolic pathways are branched and interconnected.
- Not all organisms metabolize the same compounds in the same way.
- The same kinds of reactions (i.e., acid-base, oxidation-reduction, catalyzed) common to other areas of chemistry are prominent in biochemistry.

THE ORGANIZATION OF CELLULAR ACTIVITIES

- The cell is composed of units called subcellular organelles, each of which performs a particular function in the life process.
- Membranes play not only a structural role but also an active role in cellular activity.
- Organelles may be separated on the basis of their density differences.
- The mitochondrion is the powerhouse of the cell.
- The DNA molecule in the chromosomes contains all of the genetic information for the organism.
- The information in DNA is used for the synthesis of macromolecules and is also passed on as hereditary information to daughter cells.
- A gene is a sequence of nucleotides within the DNA molecule.
- Protein synthesis results from the interaction of organelles.

WHERE ARE WE?

- Major biochemical problems remain to be solved.
- Biochemistry is vital to our well-being. The greater our knowledge of living systems, the higher can be the quality of our lives.
- The information presented in this module represents the results of a vast amount of human labor. Science is a human endeavor and it progresses only through the efforts of people dedicated to the task of unraveling the mysteries that confront us.
- Living things are complex but not beyond understanding.
- Although biochemistry has provided solutions to many problems, much remains to be done.

Module Objectives

We have attempted to group module objectives in three broad categories: concept-centered, attitude-centered, and skill-centered. The categories are not mutually exclusive; there is considerable overlap. The conditions for accomplishing each objective are not given, since they can easily be found in the respective section in the student module. Note also that concept and skill objectives are more specific than those in the affective domain. It is very difficult to classify objectives in this way, but we have been encouraged to do so by classroom teachers, who have helped in this difficult task.

The objectives identified here should provide you with a useful starting point in clarifying your own goals in teaching this module. We encourage you to identify alternative objectives, using this list as a point of departure. Assessment items can be found at the ends of major sections in the student module in the forms of *Questions, Problems,* and *Exercises.* Other *Evaluation Items* are included after each major section of this guide and in the form of module tests for knowledge and skill objectives, located in the teacher's guide appendix.

Concept-Centered Objectives	*Attitude-Centered Objectives*	*Skill-Centered Objectives*
CONSIDERING LIFE PROCESSES		
B-2 • Identify the functions of the main types of biomolecules and cite examples of them.	• Recognize that, although living things are extremely complex, they are not chemically inscrutable.	
UNDERSTANDING THE STRUCTURE OF BIOMOLECULES		
B-3 • Describe covalent bonding. • Define isomerism. **B-4** • State the bonding capacity (in numbers of bonds) of carbon, hydrogen, and oxygen. • Identify functional groups of alcohols, aldehydes, and ketones. **B-5** • Recognize carbohydrates in general by their complement of functional groups. **B-6** • Describe types of carbohydrates (mono-, di-, and polysaccharides) and give examples of each by name (not structure); for example, glucose-monosaccharide.	• Realize that our senses are avenues of information for our minds. • Recognize, in the case of unsaturated fats, the concern of biochemistry for human well-being. Also recognize the interplay of chemistry, health, and commerce.	**B-3** • Draw electron-dot and line structures of simple organic molecules. Draw isomers of simple organic molecules. **B-4** • Draw line structures for alcohols, aldehydes, and ketones. **B-6** • Classify carbohydrates on the basis of sweetness of taste. **B-9** • Draw electron-dot and line structures for the carboxylic acid, amino, and peptide groups to show how the latter is formed from the two former groups.

Concept-Centered Objectives	Attitude-Centered Objectives	Skill-Centered Objectives

B-7
- Identify, by name, carbohydrates used as energy storage compounds or used as structural compounds.
- Explain why energy storage compounds (glycogen) are necessary.

B-8
- Describe and identify the functional groups of lipids, saturated and unsaturated fats, and fatty acids and cite examples of each.
- State and describe the role of lipids and fats in living organisms.

B-9
- Identify the functional groups that make up amino acids.
- Describe the formation of the peptide linkage between amino acid functional groups.

B-6, 9
- Describe the monomer-polymer relationship for proteins and polysaccharides.

PROPERTIES AND REACTIONS OF BIOMOLECULES

B-10, 11
- Given the structure of a biomolecule, predict on the basis of functional groups present its solubility in polar and nonpolar solvents.

B-12, 13
- Describe the functional groups in each of the major classes of biomolecules (carbohydrate, protein, amino acids).

B-13
- Predict the results of classifying biomolecules with the standard tests: iodine, Benedict's, ninhydrin, and Biuret.

- Recognize the fact that chemical laws are applicable to biomolecules.
- Recognize in the zwitterion concept a model that describes observed properties of amino acids.

B-10, 13
- Distinguish between monosaccharides, amino acids, proteins, and lipids through physical and chemical tests.

Concept-Centered Objectives	*Attitude-Centered Objectives*	*Skill-Centered Objectives*

B-14
- Identify and give examples of Brønsted acids and bases.
- Define acids and bases according to Brønsted-Lowry theory.
- Explain the acidic and basic properties of amino acids.

B-15
- Identify the acidic and basic parts of a zwitterion.

ENZYMES: WHERE THE ACTION IS

B-16
- Define reaction rate.

B-16, 17
- List and describe the properties of enzymes.
- Define "active site."
- Compare properties of organic and inorganic catalysts.

B-18
- Explain, in general, how enzymes are structured.
- Explain the ways that the structures of enzymes can differ.

B-19
- Identify and describe the types of bonding found in the folded structures of enzymes.

B-20, 21
- Explain the denaturing of protein in terms of its structure.
- Cite examples of protein denaturing in daily living.

B-22
- Interpret pH values as acidic, basic, or neutral.
- Carry out calculations that relate pH with H^+ concentration and with OH^- concentration.
- Recognize that experimental results often match known conditions in the body.
- Recognize that structural models can help to explain physical realities.
- Appreciate the significance of controlling temperature in biochemical systems.

B-16, 17
- Demonstrate that enzymes are heat sensitive.
- Distinguish experimentally between catalysts and noncatalysts, and between enzymes and inorganic catalysts.

B-23
- Determine the pH of a solution, using pH paper.

B-25
- Accurately record experimental data.
- Plot reaction rate vs. pH.
- Experimentally determine the reaction rate of a given enzyme.

B-25, 27, 47
- Demonstrate the use of a pipet.

B-27
- Carry out an experiment that shows the relationship between substrate analogs and reaction rate.

B-29
- Determine the effect of temperature on reaction rate.

B-23, 25
- Cite experimental work that shows the pH dependence of enzymes.

B-21, 23, 25
- Explain why a change in pH will cause a change in enzyme activity.

B-24, 25
- Calculate rate of reaction, given experimental data.

B-25
- Convert a rate from one metric unit to another.
- Determine optimum pH for an enzyme reaction, given the experimental data.

B-26, 27
- Choose from a group of molecules one that would best fit a given active site.
- Explain what it means to inhibit the action of an enzyme.

B-28
- Explain how Hg, Pb, and other metal ions can poison a biosystem.
- Give examples, by name, of substances other than amino acids that are often in active sites.
- Explain how denaturing an enzyme affects the active site.

B-29
- Explain how temperature affects reaction rate.
- Explain how a change in temperature affects enzymatic reactions and nonenzymatic reactions.

METABOLISM: THE COMMUNITY OF ENZYME REACTIONS

B-30
- Explain and distinguish the terms *metabolism* and *digestion*.
- Match some digestive enzymes with their functions.
- Recognize the role of biochemistry in providing a knowledge of the components needed for a properly balanced diet.

B-31
- Determine if a given enzyme will hydrolyze gelatin.

Concept-Centered Objectives	*Attitude-Centered Objectives*	*Skill-Centered Objectives*
B-32 • State the main pathways of the overall metabolism of glucose. • Identify and cite examples of metabolites and cofactors. • State the function of NAD and FAD in metabolism. • Explain the functions of some vitamins in metabolism. • Explain the function of cofactors. **B-33, 36** • State the starting materials and main products of each of the main pathways of the overall metabolism of glucose. **B-33** • State the function of ATP in metabolism. **B-34** • Outline an experimental procedure for demonstrating the activity of ATP. **B-35** • State the place of the Krebs cycle in metabolism. **B-36** • Explain where most of the ATP from glucose metabolism is generated. • Explain why oxygen is so important in the metabolism of many organisms. **B-37** • Explain what is meant by branching. **B-38** • Define fermentation in terms of metabolism.	• Recognize the importance of metabolic processes in the food and pharmaceutical industries. • Recognize that not all bacteria are harmful.	**B-39** • Measure the amount of an acid by titration. • Make sauerkraut by cabbage fermentation.

THE ORGANIZATION OF CELLULAR ACTIVITIES

B-40
- Describe the main components of and their roles in a generalized cell.

B-41
- State two functions of the cellular membrane.

B-42
- From a list, select molecules that will pass through a cell membrane.

B-44, 45
- Contrast the activities of mitochondria and chloroplasts.

B-44
- Explain why the mitochondrion is called the powerhouse of the cell.

B-46, 47
- Explain how organelles may be separated by centrifugation.

B-47
- Explain the concept of density.

B-48, 49
- Define nucleotides.
- Contrast DNA and RNA molecules in terms of their components and structures.

B-50, 51
- Given a DNA sequence, be able to pair bases and "synthesize" an RNA sequence.
- Contrast the different types of RNA.
- Describe the process of protein synthesis.

- Realize the complexity of the living organism.
- Recognize that the operation of complex machines, such as kidney machines, is based on simple chemical and physical principles.
- Recognize the essentiality of photosynthesis, and hence plants, to the existence of life on this planet.
- Recognize the great human effort that went into our present knowledge of living organisms.
- Recognize that understanding the molecular basis of a genetic defect does not automatically provide a treatment or means of eliminating the defect.
- Recognize that the interdisciplinary nature of biochemical research provides a powerful means of solving problems basic to human existence.

B-42
- Perform a separation of different-sized molecules by dialysis.

B-47
- Separate particles of different densities by the density gradient method.

Teaching Molecules in Living Systems

To allow for individual preferences, we are leaving the teaching schedule and time plan up to the individual teacher. We would like you to remember, however, that a brisk pace is preferable. Because of the modular structure of the IAC curriculum, many concepts are treated several times from different points of view, and an intensive treatment every time is not necessary. Most teachers allow about six weeks teaching time for *Molecules in Living Systems*.

Considering Life Processes

This opening section of *Molecules in Living Systems: A Biochemistry Module* focuses on biomolecules in general. After a brief introduction that relates the study of biochemistry to the "real" world, the four basic types of biomolecules (carbohydrates, lipids, proteins, and nucleic acids) are listed in tabular form, along with the functions they perform in living organisms (Table 1, *Some Biomolecules and Their Biological Roles*, student module page 4).

Following a brief treatment of the carbon atom, the concept of functional groups is presented. This concept is important to the rest of the module. The carbohydrates, lipids, and amino acids (as well as proteins) are described in terms of their functional groups.

B-1 THE CHEMISTRY OF LIFE

The fundamental question, "What is life?" will provide ample matter for class discussion. Although a brief discussion is enough to set the theme for the module, you can determine the depth of your treatment—a depth that depends on your class interests. The important ideas in this section are the similarities of the activities of living organisms, the notion of "cycles," and the place of biochemistry in the scheme of things. The mutability of scientific theories is another point that could be brought out.

What's News in Biochemistry Suggest that your students review current newspapers, magazines, and other popular periodicals to look for relevant discussions of biochemistry in everyday life situations. Decide on some means of collection, discussion, and display of the materials that your students bring to class. Have them continue this activity throughout their study of *Molecules in Living Systems*. As much as possible, relate these articles to the topic area currently being discussed, or use them to review previous discussions. Suggest that students focus on the biochemical problem being investigated and determine from the article what is being done to solve this problem. Interested students may wish to do some additional research on the actual history of the problem presented in the article, if it has not already been stated. Bring out in class discussion the relevance of biochemistry to each student's everyday situation, as well as the type of research being done by researchers looking for answers to the question, "What is life?" Since it is suggested that you use this material in your summary discussion of *Molecules in Living Systems*, start the activity early in the course and plan accordingly.

B-2 FUNCTIONS OF BIOMOLECULES

This section relates to previous discussions in *Reactions and Reason: An Introductory Chemistry Module*, section *A-3*, which dealt with classifying objects into groups. The students will realize that there are many ways to group biomolecules, and perhaps they could offer some of these for consideration. This module approaches the question of grouping from the point of view of functional groups rather than that of bodily functions. This approach provides fewer basic groups and also places emphasis on the chemistry of biomolecules.

Ask students to identify the biomolecules in Table 1 with which they are familiar. It might be

a good idea to see where they first heard of biomolecules (biology class, a textbook, a magazine article, or a television program), since it will help them realize that they are exposed to biochemistry from a variety of different sources.

The list in Table 1 is not exhaustive. If your students have had a good biology background, they might be able to suggest other biomolecules as additions. Some examples are: *sucrose*, a sugar; *adrenalin*, a hormone that stimulates the heart; *ptyalin*, *pepsin*, and *rennin*, digestive enzymes; *calciferol (vitamin D)*, a lipid that is a nutrient in the body; *hemoglobin*, oxygen-carrier in blood; *gelatin*, a protein; and *cholesterol*, a lipid important in membranes and involved in cardiovascular disease. Some students may wish to look through the book for biomolecules. The *Merck Index* is an excellent source for information on biomolecules with functions that the students might know.

The definition of an enzyme is given in this section, and it should suffice until enzymes are studied later on. Students are familiar with many substances that are enzymes, but they may not have recognized them as such. An example would be meat tenderizer, which is discussed in more detail in section *B-31*.

Miniexperiment As an extension to a previously suggested activity, suggest to your students that they focus on the articles from newspapers which relate to foods and medicine. Have students summarize their articles in a class discussion. Just reading articles from food sections and medical columns can be informative and profitable, but it would be of even greater value if students were to analyze and draw specific bits of information from the articles. For example, after reading an article on carbohydrates, the following questions could be asked: "From what you have learned by reading *Molecules in Living Systems*, would you consider the newspaper article accurate? If not, where are the errors? Name the biomolecules mentioned in the article. To which class do they belong?" This activity can be continued throughout the module.

Understanding the Structure of Biomolecules

B-3 THE AMAZING CARBON ATOM

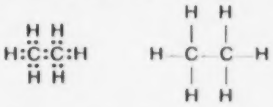
Carbon, although it is not very plentiful in nature (less than 0.1 percent by mass of the earth's crust) forms a vast number of different compounds because of its ability to form stable bonds not only with itself but with many other atoms as well. This is a good time to review the writing of Lewis dot formulas as well as the line structures by giving students examples involving carbon. This will make it easier for them to recognize the structural diagrams that are drawn in this module.

Reactions and Reason: An Introductory Chemistry Module and *Form and Function: An Organic Chemistry Module* give information on bonding, valence electrons, and three-dimensional structure. Using molecular models (or ball-and-stick models), students will be able to picture the three-dimensional structure of the molecules discussed in the student module text. The concept of three-dimensional structure, but not optical isomerism, will be extremely important in understanding many aspects of this module. The models can also be used to expand on the concept of isomers, although we do not discuss this subject in great detail.

Miniexperiment *Building Molecular Models:* You may wish to have your students try working with molecular models by using inexpensive kits produced by Lab-Aids, Inc. (130 Wilbur Place, Bohemia, NY 11716). The atoms in this kit consist of plastic nuclei with pegs set at correct bond angles to produce accurate three-dimensional models. The kits contain separate procedures, worksheets, and a guide prepared by the company. There is enough material for up to 50 students and the materials can be used again and again. Some of the kits center around proteins, fats, carbohydrates, functional groups, and hydrocarbons. Write to the company for price list and description. You may wish to use this type of kit in sections *B-4*, *B-5*, *B-6*, and *B-27*.

ANSWERS TO EXERCISES

(Student module page 7)



B-4 FUNCTIONAL GROUPS: KEY TO REACTIVITY

Since the functional group concept is the foundation of the module's treatment of biomolecules, emphasize the importance of learning to recognize these groups in a molecule. Again, Lewis dot structures can be used to show the structure of the functional groups. Page 10 of the student module concludes the discussion of functional groups. For further practice in reviewing these groups, you may wish to have your students try the following activity.

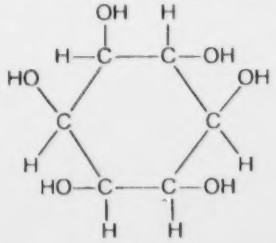
Miniexperiment *Functional Groups:* Below are molecular structures that contain functional groups that you have just discussed. Ask your students to identify and name each functional group and the compound. You may wish to present these by duplicating copies of the structures or by placing examples on the chalkboard. In any case, this list is not exhaustive; additional structures for discussion are abundant.

To continue this activity, ask students to write the molecular formulas (or match the ones that you provide) to the molecular structures. The following match the structures above: CH_2O; CH_4O; C_2H_4O; C_2H_6O; C_3H_6O; C_3H_8O; C_4H_8O.

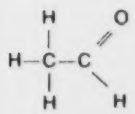
Possible Answers:

CH_2O—formaldehyde
CH_4O—methyl alcohol (methanol)
C_2H_4O—acetaldehyde
C_2H_6O—ethyl alcohol (ethanol)
C_3H_8O—propyl alcohol (propanol)
C_3H_6O—acetone, propionaldehyde
C_4H_8O—methylethyl ketone

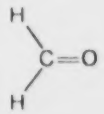
Discussion of questions on page 11 of student module: How many different structures of $C_6H_{12}O_6$ can you come up with? How do you know they are different? Some examples of these will be introduced in the following section, *B-5*, but encourage students to try this activity before they proceed with this discussion. Students will devise structures other than the ones presented in the following section. One example could be inositol, which is also a biomolecule.



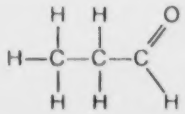
inositol



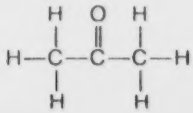
acetaldehyde



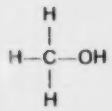
formaldehyde



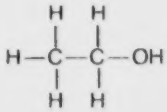
propionaldehyde



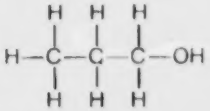
acetone



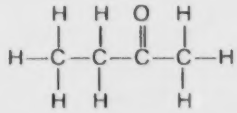
methanol



ethanol



propanol



methylethyl ketone

(Two aldehyde structures are also possible here.)

It is best at this point not to try to make this exercise an exhaustive one, since this could lead to the topic of optical isomerism. The main idea is to show the great variety of isomers one can draw for a molecule with the formula $C_6H_{12}O_6$. You could ask students to classify all of the above substances according to functional groups (aldehydes, alcohols, ketones, acids) to emphasize the concept.

B-5 CARBOHYDRATES

This section provides the student with the opportunity to recognize several functional groups within the same molecule. Those students who have difficulty in recognizing the groups in the carbohydrate molecules whose structures are shown on pages 11 and 12 of the student module should review the previous section. Some carbohydrates (e.g., glucose, on page 12) are represented in a useful, stylized manner called Haworth structures. Students will not be troubled by them; most biochemistry tests explain Haworth structures (e.g., John W. Suttie, *Introduction to Biochemistry*, 2nd ed. [New York: Holt, Rinehart & Winston, 1977].)

The question of the difference between glucose and galactose may arise, since they appear to be similar in structure. The difference is steric; the best way to show how the two molecules are different would be to build a model of each in cyclic form and to show that they cannot be superimposed.

MINIEXPERIMENT
B-6 HOW SWEET IT IS

The purpose of this miniexperiment is to show that small differences in structure can be important biologically.

Concept In doing this experiment, students will encounter this important idea:*

- Small changes in structure may have a great influence on biological activity.

*This statement appears only with this first experiment, but it applies each time this section appears in an experiment, unless otherwise noted.

Objective After completing this experiment, a student should be able to:*

- Determine relative sweetness.

Estimated Time 20 minutes

Student Grouping Individuals

Materials*

6 plastic spoons
3.0 g each of the following sugars:
  glucose
  galactose
  fructose
  sucrose
  maltose
  lactose
30 cups, plastic *or* paper

All of the sugars can be purchased from biological supply houses such as: Calbiochem-Behring-Corp. (10933 North Torrey Pines Road, La Jolla, CA 92037); Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178).

Lactose and glucose (dextrose) can also be obtained from local sources, such as pharmacies.

Lactose is often referred to as milk sugar; it is the major carbohydrate in milk. Glucose, fructose, and maltose are also known as dextrose, levulose, and maltobiose, respectively.

Advance Preparation Each student will need approximately 0.1 g of each sugar for testing. You may wish to dispense these on labeled paper squares.

Prelab Discussion It is very important to emphasize that indiscriminate tasting of chemicals is definitely forbidden in the lab. Students should be made aware of the fact that this experiment is an exception to a rule that should be followed in the lab: *Do not taste chemicals.*

To help students get better results, remind them to rinse their mouths with water between tastings.

Range of Results The table given below is meant only to represent a general trend of results for this

*The *Materials* list for each laboratory experiment in this module is planned for a class of 30 students working in pairs, unless otherwise specified. You may have to adjust this to fit the size of your class.

experiment. The class, as a whole, will probably agree with these results.

		Relative Sweetness		
Compound	*Molecular Formula*	*Very Sweet*	*Medium Sweet*	*Not Sweet or Slightly Sweet*
Glucose	$C_6H_{12}O_6$		X	
Galactose	$C_6H_{12}O_6$			X
Fructose	$C_6H_{12}O_6$	X		
Sucrose	$C_{12}H_{22}O_{11}$	X		
Maltose	$C_{12}H_{22}O_{11}$		X	
Lactose	$C_{12}H_{22}O_{11}$			X

Postlab Discussion Construct a blank table on a chalkboard or overhead transparency so that the class can pool its results. There will be some disagreements, since taste is a subjective measurement. This is a good example of an experiment that gives varying results, all of which can be said to be "right."

The results should show a difference between the relative sweetness of glucose and galactose. By comparing this result to the structural diagrams or models of these two molecules you can demonstrate the point of the experiment. Small differences in structure make a big difference in activity. As for the discussion of mono-, di-, and polysaccharides, you can carry it as far as the interest and level of your students dictate. If you wish to expand the discussion, add starch, a nonsweet polysaccharide, to the list of carbohydrates being tested.

Another method of classifying carbohydrates is on the basis of whether they contain either an aldehyde or a ketone group. Those that are aldehydes are called *aldoses*, and those that are ketones are called *ketoses*. Each of these is further classified according to the number of carbon atoms in the structure. Five carbons would have "pent" in the name. Six carbons would be "hex." According to this system, the following names would be used:

glucose—aldohexose
galactose—aldohexose
fructose—ketohexose

You can see that this system does not discriminate between the various sugars. The aldose-ketose system is only a means of naming general groups, but it has the advantage of describing the structure by naming it.

For students interested in finding out more about theories of sweetness and tasting, there are a number of articles in the literature. (For example, see *Journal of Chemical Education* [March 1972], p. 171.) One of the most widely accepted theories correlates sweetness with the degree and type of intramolecular hydrogen bonding among adjacent groups, such as the polar —OH groups. In order to achieve hydrogen bonding, these —OH groups must have the correct orientation relative to each other.

B-7 CARBOHYDRATES AS ENERGY COMPOUNDS

Sections *B-30* through *B-39* treat metabolism in greater detail, so in this section the treatment should be kept on a very general level. The main point is to give the student an idea of how carbohydrates are used by the body. Stress the fact that there is more to what goes on inside our bodies than one might suspect.

You may wish to show students the different bonding that exists in starch and cellulose, using molecular models or overlay transparencies. However, this involves a difference in stereochemistry once again. On the other hand, the difference between starch and glycogen is the degree of branching. This is not important for understanding the rest of the module, and the concept thus is not introduced in the student module. It is not intended that stereochemistry should be discussed, but if students cannot understand the differences in the structures, you can relate them to the differences between glucose and galactose. The differences in structure between starch and cellulose makes a big difference to enzymes that break down these materials, just as the differences in the structures of glucose and galactose affected the sweetness of the compounds.

The inability of the body to digest lactose is common after weaning in most of the countries of the world. In these countries, milk is not a common food for adults. This inability to digest lactose results from a decrease and finally a cessation of the production of the enzyme needed to break down lactose. The cramps and diarrhea associated with this enzyme deficiency probably result from bacterial fermentation of the unabsorbed lactose and from excess water in the intestine. The latter can be explained by the osmotic pressure created by the undigested lactose in the intestine. The intestinal contents will retain

enough water to maintain a pressure equilibrium with the body. Thus, the more lactose, the greater is the amount of water in the intestine. (See N. Kretchmer, "Lactose and Lactase," *Scientific American* [October 1972], pp. 70–78.)

You may want to mention that research in which cattle are being fed a steady diet of urea and cellulose is being conducted by the United States Department of Agriculture. Microbes are present in the digestive tract of these animals. The microbes contain an enzyme capable of digesting cellulose. This is significant research in that underdeveloped countries that cannot afford cattle feed may, in the future, be able to fatten their herds of cattle by feeding them a diet composed chiefly of "sawdust."

B-8 LIPIDS: ANOTHER SOURCE OF ENERGY

The fatty acid structural formulas may seem formidable to the student, but the important features are the carboxylic acid functional group at one end of the molecule and the long hydrocarbon chain devoid of functional groups. It is the carboxylic acid group that is responsible for the formation of triglycerides, which, like carbohydrates, are energy storage compounds. The notion of polyunsaturated molecules is also treated, since it is applicable to everyday living and illustrates the impact of science on improving the quality of our lives.

In their study of *Form and Function: An Organic Chemistry Module*, students use fats and oils to make soap (experiment *O-42 Preparation of Soap*). Some of your students may therefore be familiar with this aspect of lipid chemistry. The most familiar use of fats and oils is dietary. A table of information on such edible oils follows.

Lard, shortenings, and oils are essentially all lipid. The distinction between an oil and a fat is based on the "melting point" of the product. Fats are solids at room temperature, while oils are liquids.

Cooking and salad oils are composed of about 98 percent triglycerides. The remainder of the oil is sterol, free fatty acid, and pigments (such as carotene). Traces of antioxidants are added to all but olive oil. Olive oil contains a natural antioxidant. Olive oil is the only product that is marketed immediately after pressing the oil (from the fruit of the evergreen tree *Olea europea*) from the olives. All other oils must undergo processing to decolor and deodorize the product before marketing.

Lard is rendered pork fat and contains about 99 percent triglycerides, the remainder being free fatty acids and probably cholesterol.

FATTY ACID COMPOSITION OF SOME IMPORTANT COMMERCIAL FATS AND OILS[1]

(mass percent)

Fatty Acid	*Butter*	*Margarine*[2]	*Lard*	*Olive Oil*	*Safflower Oil*	*Peanut Oil*	*Corn Oil*	*Linseed Oil*
saturated	**70**	**22**	**45**	**16**	**10**	**17**	**17**	**10**
C_4–C_{10}	11	—	—	—	—	—	—	—
C_{12}–C_{14}	19	t	3	t	—	t	t	—
C_{16}–C_{18}	40	22	42	16	10	11	17	10
C_{20}–C_{24}	t[4]	t	t	t	—	6	6	t
unsaturated[3]	**30**	**78**	**55**	**84**	**90**	**83**	**83**	**90**
18:1	20	61	42	64	13	61	29	20
other monoenes	7	t	4	2	—	—	—	—
polyunsaturated	3	17	9	16	77	22	54	70
18:2	3	17	9	16	76	22	54	16
18:3	t	t	—	—	1	—	—	54

[1]All figures are for edible oils, except for linseed oil, which is used as a drying oil in paints and similar products.
[2]The margarine data are for a sample that was composed of partially hydrogenated cottonseed and soybean oils.
[3]The number before the colon indicates the chain length, and the number after the colon designates the number of double bonds in the fatty acid. In most fats and oils the 18:1 is oleic acid, 18:2 is linoleic acid, and 18:3 is linolenic acid.
[4]t indicates trace amounts.

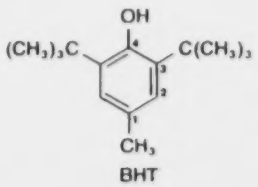
Shortenings are fats (usually made from partially hydrogenated vegetable oils) to which 1–20 percent by mass of emulsification agents have been added. Emulsification agents usually added are monoglycerides and diglycerides.

Butter is 80 percent fat (milk fat) and greater than 95 percent of this is triglyceride. Butter also contains 1 to 5 percent air, 1.5 to 3.0 percent salt, and about 1 percent milk solids, such as casein. The remainder is water. Other milk fat lipids (phospholipids, monoglycerides, and diglycerides) confer good emulsification properties on butter. Margarine is prepared from partially hydrogenated oils and other compounds to imitate the properties of butter.

Most liquid hydrocarbons (such as hexane, cyclohexane, and benzene), as well as some halogenated hydrocarbons (such as chloroform) are suitable for use as lipid solvents. The main requirement for such a solvent is that it be quite nonpolar with respect to water or methanol.

Ask students to read the labels of some cooking oils and spreads that are in their kitchens to see if they recognize terms that have appeared in this section. This activity will help illustrate that chemistry is not limited to the classroom.

Many products will have additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These are antioxidants added to minimize oxidation of the double bonds in the fatty acids. The antioxidants are oxidized instead. BHA is similar, except it has an OCH_3 group in position 4.



BHT

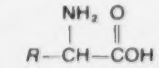
Miniexperiment Some margarines are advertised as low-calorie products. The caloric content is lowered by adding more water. Students can test for the water content in such products by placing a small amount in a small graduated cylinder, heating the contents *in a water bath*, and measuring the volumes of the water and oil layers formed when the margarine melts.

Vitamin E is a natural antioxidant. It has been added to deodorants to help prevent the oxidation of lipids present in skin oils. Many products of the oxidation of lipids smell bad.

B-9 PROTEINS AND AMINO ACIDS

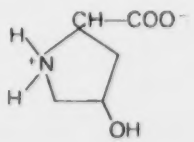
Proteins and enzymes are treated extensively in this module, and thus this section is basic to understanding the structures of these molecules. The amino acids are treated in terms of functional groups, which, in turn, are used to explain a very fundamental biochemical reaction: the formation of peptide linkages.

You might illustrate for the students that the basic structure of the amino acid can be represented as follows:

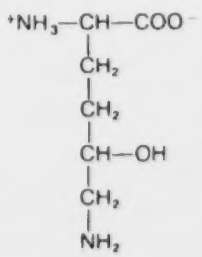


The only difference among the amino acids is the structure of the "R" group. Note that the amino group is attached to the carbon atom next to the carboxyl group (alpha position). Therefore, amino acids are often called α-amino acids. We have shown the structures of 20 amino acids in an appendix to the student module. A few other amino acids occur in some special proteins. For example, collagen contains hydroxylysine and hydroxyproline. These amino acids are not incorporated into the protein as the peptide chain is formed, but result from the hydroxylation of the proline and lysine that are in the already-formed molecule.

HYDROXYPROLINE



HYDROXYLYSINE



We do not want students to memorize the structures of all the amino acids. Rather, they should be able to recognize the fundamental components that make up amino acids. Through

repeated exposure, they will learn some of the more common amino acids.

Many students realize that human beings ingest proteins in the form of meats such as steaks, chops, and hamburgers. Remind your students that protein is also available in nonmeat products *such as beans, eggs, fish, soybeans, oatmeal,* peanut butter, wheat germ, and cottage cheese.

You may wish to point out in class that the reaction which links many amino acids together to form a protein is essentially a polymerization reaction (see *Form and Function: An Organic Chemistry Module*, section *O-47 From Monomer to Polymer*). Protein is a natural polymer of amino acid monomers. The reaction is similar to the ones that produce synthetic polymers such as nylon. Although not mentioned in the student module, a similar monomer-polymer relationship exists between glucose and the polysaccharides starch, glycogen, and cellulose.

EVALUATION ITEMS

These are additional evaluation items that you may wish to use with your students at various times during the preceding section. The correct answer to each question is indicated by shading.

1. Four classes of biomolecules are: A. *carbohydrates*, B. *nucleic acids*, C. *proteins*, and D. *lipids*. Give as many examples of each of the four classes as you can.

   See Table 1 student module page 4 for the possible answers.

2. Cellulose belongs to which major class of biomolecules? carbohydrates

3. We can differentiate between classes of biomolecules on the basis of their biological function. T or F

4. In the family of biomolecules there are organizers, talkers, doers, and regulators. Which of the following would be considered a regulator?

   A. starch B. insulin C. trypsin D. maltose

5. Proteins perform all of the following functions except:

   A. structure and organization
   B. catalysis
   C. control and regulation
   D. reproduction and information storage

6. Draw two possible isomers for the molecule that has the formula C_4H_{10}.

   $CH_3—CH_2—CH_2—CH_3$

   $CH_3—CH(CH_3)—CH_3$

7. The word that is most closely associated with the term *covalent* is:

   A. valence C. taking E. sharing
   B. giving D. combining

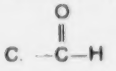
8. On the basis of valence electrons, explain why the CH_4 molecule is stable, while the CH_3 molecule is not.

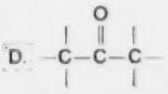
   Each carbon atom has four valence electrons and requires a share of eight valence electrons to form a stable compound.

9. Knowing that a compound is a ketone, we can safely conclude that it contains the following:

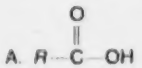
   A. —NH

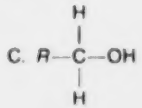
   B.

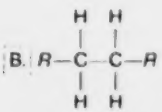
   C. 

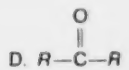
   D. 

10. Of the structures given, select the one that does *not* contain a functional group.

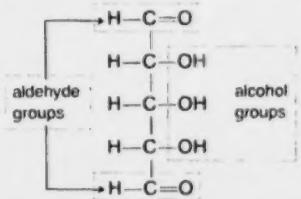
    A. 

    C. 

    B. 

    D. 

11. In the structure given below, circle each functional group and give its name.

12. Show how aldehydes and ketones differ by drawing their line structures.

    See student module page 10.

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13. Is the cyclic structure or the linear structure of glucose more common in biological systems?

the cyclic structure

14. Into what three general classes can we divide carbohydrates?

monosaccharides, disaccharides, and polysaccharides

15. The following are carbohydrates:

A. glucose C. maltose E. sucrose
B. starch D. fructose F. cellulose

Of those listed, which are monosaccharides?
A, D

16. Of the following, which is not a polysaccharide?

A. fructose B. glycogen C. starch D. cellulose

17. In the body, as glucose is broken down, the metabolic energy is conserved in:

A. glycogen B. CO_2 C. ATP D. insulin E. H_2O

18. A storage form of glucose is:

A. glycogen D. maltose
B. fat E. carbohydrate
C. ATP

19. Place the following items in sequence of digestion and storage of carbohydrates:
(a) starch, (b) intestinal enzyme, (c) glycogen, (d) glucose, (e) saliva, (f) maltose

a, e, f, b, d, c

20. Why is the formula for palmitic acid written as $C_{15}H_{31}COOH$ instead of $C_{16}H_{32}O_2$?

The former structure emphasizes that palmitic acid has a carboxylic acid group.

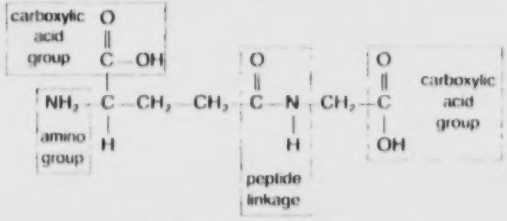
21. A distinctive and characteristic functional group of fats is:

A. a ketone group. C. a peptide group.
B. a —CH— group. D. an ester group.

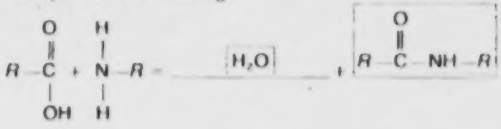
22. Which of the following is not true about triglycerides and proteins?

A. They both constitute major groups of biomolecules.
B. Both contain amino functional groups.
C. They are both hydrolyzed in order to enter the metabolic pathway of the human organism.
D. They both contain derivatives of the functional group, carboxylic acid.

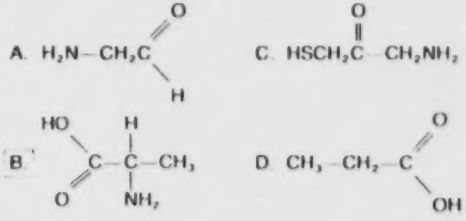
23. Circle and identify the functional groups and the peptide linkage(s) in the following molecule:



24. Complete the following:



25. Which of the following is an amino acid?



Properties and Reactions of Biomolecules

A major purpose of this section is to lay a foundation for understanding the reactivity and properties of biomolecules. Many of the concepts developed in this section will be important to a full understanding of the structure and function of enzymes, which will be discussed in the next major section.

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EXPERIMENT
B-10 SOLUBILITIES OF BIOMOLECULES

The purpose of this experiment is to observe the differences in the solubility of four biomolecules in polar and nonpolar solvents.

Concepts

- Biomolecules that contain many functional groups are polar and therefore will be soluble in polar solvents, such as water.
- Biomolecules that contain few or no functional groups are usually nonpolar and therefore are soluble in nonpolar solvents, such as hexane.

Objectives

- Predict whether a molecule will be soluble in a polar or nonpolar solvent, given its structure.
- Differentiate among proteins, sugars, amino acids, and lipids, given a series of structural formulas for biomolecules.
- Use the correct amount for testing purposes.

Estimated Time 30 minutes

Student Grouping Pairs

Materials

60–80 18 × 150-mm test tubes
15 test-tube racks
15 spatulas
750 cm^3 hexane *or* Du Pont TF Solvent (see *Advance Preparation*)
7.5 cm^3 vegetable oil
7.5 g glucose
7.5 g monosodium glutamate
7.5 g gelatin

Advance Preparation The glucose, gelatin, and monosodium glutamate may be supplied in small beakers or paper cups. The vegetable oil may be dispensed from small dropper bottles. Some teachers have found that Nalgene dropping bottles work well. If burets with Teflon stopcocks are available, they could be used to deliver the hexane directly into the test tubes.

Instead of hexane, you may use a nonpolar solvent such as Du Pont TF Solvent (technical-grade trichlorotrifluoroethane). Monosodium glutamate (MSG), a flavor enhancer, is available in the condiment section of your grocery store. Many persons react to excess MSG in the diet with a variety of symptoms that have been collectively referred to as the "Chinese-restaurant syndrome," since many Chinese foods are prepared with this condiment. MSG was formerly added to baby foods; however, because of questions regarding its safety, its use is no longer allowed in the preparation of baby foods.

We recommend *unflavored, uncolored* gelatin for all the experiments requiring gelatin in this module. Plain gelatin is readily available from a local grocery store.

Prelab Discussion You may feel that it is necessary for your class to review the difference between polar solvents and nonpolar solvents. This topic was covered in section *A-45* of *Reactions and Reason: An Introductory Chemistry Module*. Students should be reminded that hexane and most other organic solvents are volatile, flammable substances.

Range of Results The solubilities of the biomolecules are shown in the table below.

Biomolecule	*Soluble in Water*	*Soluble in Hexane*
glucose	yes	no
monosodium glutamate	yes	no
gelatin	partially	no
vegetable oil	no	yes

Postlab Discussion Have students report their results and compare data with classmates. There are four observations a student will make from this experiment.

1. The lipid substance (vegetable oil) is insoluble in water but soluble in hexane.
2. The carbohydrate (glucose) and amino acid (monosodium glutamate) are soluble in water but insoluble in hexane.
3. Gelatin, even though a polar molecule overall, is only partially soluble in cold water. This is due to another factor that affects solubility: large molecular weight. Gelatin dissolves readily in hot water.
4. Hexane does not dissolve in water.

B-11 LIKE DISSOLVES LIKE

This section is actually a postlab discussion of the previous experiment. Students should be

able to see that in general the solubility of molecules can be predicted from the "rule" that like dissolves like. Point out the meaning of the terms *hydrophobic* and *hydrophilic*. These will be used later in section *B-19*.

ANSWERS TO EXERCISES

(Student module pages 29–30)

1. The term *hydrophobic* refers to substances that are insoluble in water, whereas the term *hydrophilic* refers to substances that are soluble in water.
2. Since vitamin A is mostly hydrocarbon it is soluble in hexane but insoluble in water. Vitamin A is a lipid, and it is hydrophobic.
3. Lysine is an amino acid. It has three functional groups: two amino groups and one carboxylic acid group.
4. Since vitamin C contains a large proportion of functional groups, it is soluble in water but insoluble in hexane. Vitamin C, also known as ascorbic acid, is a carbohydrate.
5. Hexane is a nonpolar solvent because it contains no functional groups.

B-12 IDENTIFYING BIOMOLECULES

This section sets the stage for the next experiment. It raises the question of how to identify biomolecules that have similar solubility properties. Once again, stress is placed on the importance of functional groups in determining the chemistry of biomolecules.

Some students may realize that there must be at least one aldehyde on each molecule of starch. This is true, in fact, but only one in several thousand glucose molecules has an end group with a free aldehyde group. The chemical tests we have chosen are not sensitive enough to determine this small amount of an aldehyde.

EXPERIMENT
B-13 CHEMICAL REACTIONS OF BIOMOLECULES

This experiment reinforces the idea that functional groups play an important part in the study of biomolecules. It points out one method chemists use to pursue the important task of identifying biomolecules. The purpose of the experiment is to identify biomolecules on the basis of their reactions with test reagents.

Concepts

- Different biomolecules contain characteristic reactive groups called functional groups.
- Functional groups undergo specific chemical reactions.
- In some cases, the test reaction cannot be explained by the chemistry of the individual functional groups; the Biuret and iodine tests require specific macromolecular superstructures.
- Reactions with test reagents frequently can be used to identify functional groups and the general class of biomolecule.

Objective

- Identify selected biomolecules by the results obtained with test reagents.

Estimate Time *Part A*, 50 minutes; *Part B*, 20–30 minutes

Student Grouping Pairs

Materials

60–120 18 × 150-mm test tubes
15 test-tube racks
15 150-cm³ beakers
15 ring stands and rings
15 clamps, universal
15 wire gauze, asbestos centers
15 Bunsen burners
75 cm³ glucose solution (1%)
75 cm³ monosodium glutamate solution (1%)
75 cm³ gelatin solution (1%)
75 cm³ starch solution (1%)
30 cm³ iodine reagent
75 cm³ Benedict's reagent
75 cm³ ninhydrin solution (1%)
75 cm³ pyridine solution (10%)
75 cm³ Biuret reagent
10 burets, clamps, and stands for solutions

Advance Preparation Prepare the solutions in advance. Some of them will be used in the experiments in sections *B-23* and *B-42*, so you may want to prepare enough for those sections also. The biomolecule solutions may become moldy if kept too long, but the others are stable solutions.

Some of the solutions will also be used as unknowns.

Monosodium glutamate: 1 percent—dissolve 1 g monosodium glutamate in 99 cm^3 distilled water.

Gelatin: 1 percent—dissolve 1 g gelatin in 99 cm^3 distilled water.

Glucose: 1 percent—dissolve 1 g glucose in 99 cm^3 distilled water.

Starch: 1 percent—make a paste of 1 g soluble starch with 3 cm^3 distilled water, and then add 96 cm^3 distilled water and heat until the solution is complete. Be sure to stir the solution continuously during the heating period.

Ninhydrin: 1 percent—dissolve 1 g ninhydrin in 99 cm^3 distilled water.

Pyridine: 10 percent—add 10 cm^3 pyridine to 90 cm^3 distilled water. This should be carried out in a fume hood or well-ventilated area, since pyridine has a very offensive odor.

Iodine reagent: Dissolve 20 g KI in distilled water, add 10 g I_2 with stirring, and dilute to 1000 cm^3.

Biuret reagent: Dissolve 1.5 g $CuSO_4 \cdot 5H_2O$ and 6 g sodium potassium tartrate in 500 cm^3 distilled water. Add to this solution, with thorough mixing, 300 cm^3 10 percent NaOH that contains a 1 g KI. Dilute this solution to 1000 cm^3.

Benedict's reagent: Dissolve 173 g sodium citrate and 100 g anhydrous sodium carbonate in 850 cm^3 distilled water. Add to this solution, with constant stirring, a solution of 17.4 g $CuSO_4 \cdot 5H_2O$ in 150 cm^3 distilled water.

If possible, all of the above reagents should be placed in burets so students can deliver the required volumes directly into test tubes. **Caution:** *Remember that pyridine, because of its offensive odor, should be set up in a hood or a well-ventilated area of the laboratory.* Evaporation of water from the tip of the buret may cause clogging, especially with the starch solution.

Prelab Discussion **Caution:** *Point out that pyridine is a flammable substance and as such should be treated accordingly. It is also a possible carcinogen, so students should avoid inhaling its vapors. Also warn students to be careful with ninhydrin. It is an irritant to skin, eyes, and nose, and can stain skin and clothing.*

To explain the "why" of this experiment, you could point out to students that before biochemists can begin to study the functions of an organism they must first identify the constituents of that organism. The tests that students will run, however, represent only a very small portion of the arsenal that biochemists have at their disposal. Spectroscopy plays a very important role, and some students might profit from doing some library research on this topic.

During the experiment, as you move around the lab, it would help to ask students to interpret the results they get from the various tests. ("What does the blue color indicate?") This will keep the experiment from becoming a "cookbook exercise."

Range of Results

Sample	*Iodine test*	*Benedict's*	*Ninhydrin*	*Biuret*
monosodium glutamate	–	–	+	–
gelatin	–	–	+	+
glucose	–	+	–	–
starch	+	–	–	–

Explanation of tests:

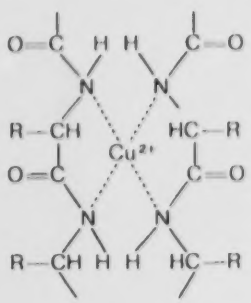
1. *Iodine test*—This color change is the result of a complex between I^-, I_2, and starch. The iodine is thought to be trapped in the helical structure of the starch. If the starch solution is too warm, the helical structure will not exist and no complex will form.

2. *Benedict's test*—This is a redox reaction.

$$Cu^{2+} + OH^- + \text{sugar} \longrightarrow \underset{\text{(red)}}{Cu_2O} + \text{oxidation products}$$

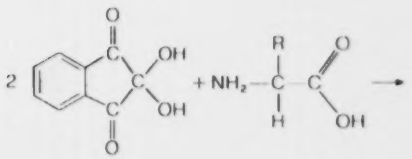
   An aldehyde group is required to reduce the Cu^{2+} ion. For this reason these sugars are called "reducing sugars."

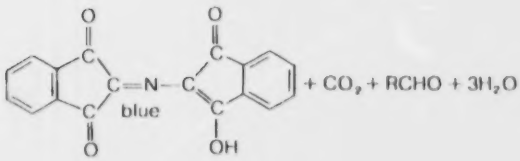
3. *Biuret test*—The purple color results from a coordination complex formed between Cu^{2+} and four peptide nitrogen atoms. Therefore only proteins give this color.



Since Biuret reagent contains Cu^{2+}, it may react with other compounds (i.e., glucose), especially when heated.

4. *Ninhydrin test*—The blue color results from compounds formed when ninhydrin reacts with an amino acid.



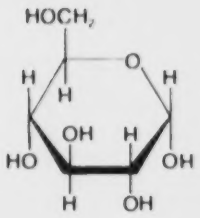


Thus, any compound with an amino group will give a color with ninhydrin, and positive tests will be obtained with *both proteins and amino acids*.

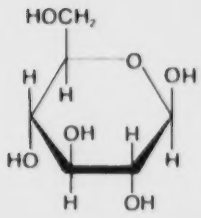
Postlab Discussion Some students, once they have performed the experiment, may wish to see if the tests can be applied to certain foods. If possible this should be done, even though the results may not be conclusive because of the heterogeneity of certain foods and the interfering reactions that result from these complexities. The topic of coordination compounds is treated in *Diversity and Periodicity: An Inorganic Chemistry Module*. Perhaps some of your students might be interested in this aspect of the experiment.

Students could be asked to describe a procedure that would allow them to identify positively any of the four groups found in this experiment by using only *two* tests. The solution is first to use the ninhydrin test, and, if it is positive, then the Biuret test will tell whether the sample is an amino acid or a protein. If the ninhydrin test is negative, then either the iodine or Benedict's test will discriminate between starch and glucose.

Glucose units can exist in either the alpha (α) or beta (β) forms, as shown below. This has not been included in the student module for reasons of simplicity and also because we did not feel that this concept was important to the discussion of chemical tests for functional groups.



α-GLUCOSE



β-GLUCOSE

Free glucose exists in both alpha and beta forms because of the equilibrium between the cyclic and open structures. Of course, this is not true of starch (alpha linkage) or cellulose (beta linkage) because there is no equilibrium since the hexose units are permanently held in the cyclic form.

Answers to questions

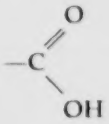
1. Egg white is a concentrated protein solution and should give a positive result in the ninhydrin and Biuret tests.
2. No. If the iodine test were sensitive to the alcohol group, then it would give positive results for glucose that contains these groups.

B-14 AMINO ACIDS: BASIC AND ACIDIC FACTS

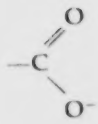
B-15 ZWITTERIONS: NEGATIVE AND POSITIVE

These sections conclude the general treatment of the types of biomolecules. The Brønsted-Lowry theory of acids and bases is employed. In this section we have avoided the use of $H^+(aq)$ since this convention does not aid the beginning student in understanding the concept of acids and bases.

To emphasize the ionic forms and the acid-base relations, we have used the terms *carboxylic acid* for



carboxylate ion for



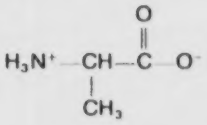
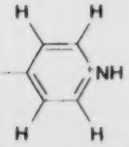
amino for (—NH_2), and *ammonium* for (—NH_3^+).

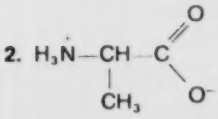
This terminology is carried on throughout the section on zwitterions. However, later in the student module we refer to carboxylate and carboxylic acid groups collectively as carboxyl groups.

ANSWERS TO EXERCISES

(Student module page 37)

1.

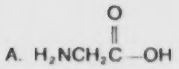
	Acid	*Base*
a.	HNO_3	CH_3NH_2
b.	HCl	H_2O
c.	H_2O	S^{2-}
d.	H_2SO_4	
e.		OH^-
f.	$NH_3—CH_2—COOH$	$CH_3—NH—CH_3$

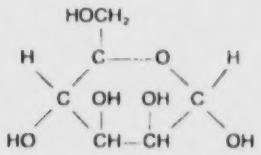
2. 

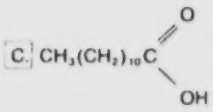
EVALUATION ITEMS

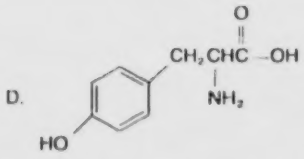
These are additional evaluation items that you may wish to use with your students at various times during the preceding section. The correct answer to each question is indicated by shading.

1. Which of the following would most likely be insoluble in water?

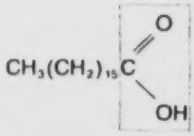
A. 

B. 

C. 

D. 

2. Circle the hydrophilic portion of the following molecule.



3. A certain compound tested positive with ninhydrin and negative with Biuret. The compound is

A. a reducing sugar.
B. a fat.
C. a protein.
D. an amino acid.

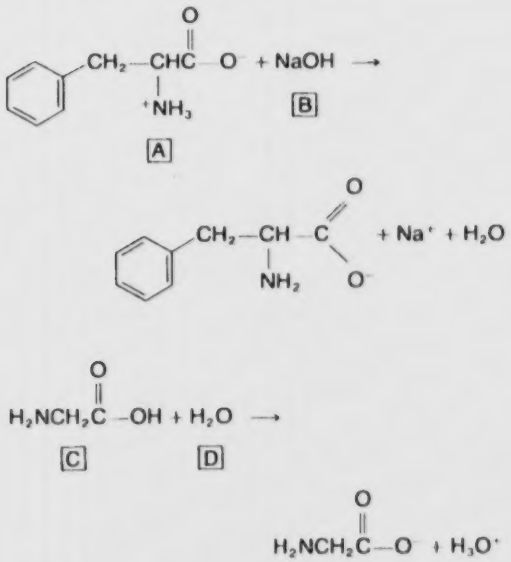
4. Another compound tested negative with ninhydrin and positive with Benedict's solution. This compound is

A. a monosaccharide.
B. a lipid.
C. a protein.
D. an amino acid.

5. Amino acids usually exist in the form of zwitterions. This means that

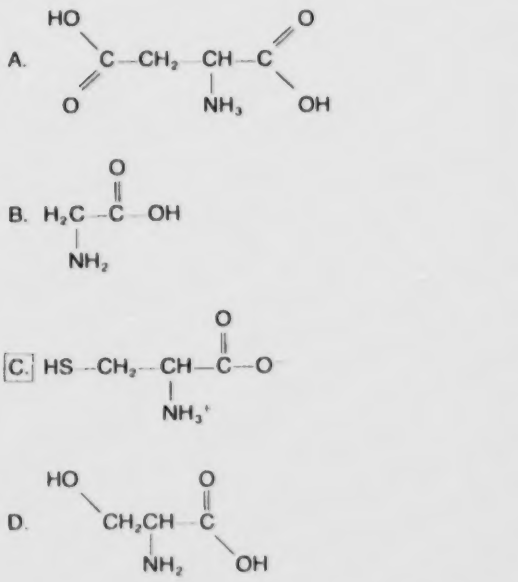
A. the basic group is the —NH_2 and the acidic group is the —COOH.
B. the basic group is the —NH_3^+ and the acidic group is the —CO_2^-.
C. the basic group is the —CO_2^- and the acidic group is the —NH_3^+.
D. they do not have basic or acidic groups as such.

6. In the reactions below, those compounds that act as acids are:



[A] A and C
B. A and D
C. B and C
D. B and D

7. Which of the following structures represents a zwitterion?



Enzymes: Where the Action Is

This part of the module deals entirely with enzymes because they represent a very important field of study for the biochemist. The purpose of this part of the module is to see what enzymes are, what they do, and how they are affected by pH and temperature. There is a fair amount of theoretical material presented here, but we have tried to keep the presentation experiment-oriented.

MINIEXPERIMENT
B-16 CATALYSTS AND REACTION RATES

We begin by showing the catalytic nature of enzymes. To do this, we compare enzymes with inorganic catalysts. The heat lability of enzymes is also shown, although this is treated more fully in later sections. The purpose of this miniexperiment is to introduce two characteristic properties of enzymes: they are heat sensitive, and they increase the rate of a chemical reaction.

Concepts

- Enzymes are catalysts and therefore can increase the rate of a chemical reaction.
- Enzymes are biomolecules (proteins), and, therefore, their catalytic properties can be destroyed by boiling.

Objectives

- State the function of a catalyst.
- Compare and contrast the properties of inorganic catalysts and enzymes.
- Show experimentally that enzymes are heat-senstive.

Estimated Time 20 minutes

Student Grouping Pairs

Materials

15 150- to 400-cm^3 beakers
15 test-tube racks
75 18 × 150-mm test tubes
15 medicine droppers
15 spatulas
15 Bunsen burners
15 ring stands and rings
150 cm^3 3 percent hydrogen peroxide (in dropper bottles)
7.5 cm^3 liver juice (blood)
1.5 g NaCl
1.5 g MnO_2

Advance Preparation If fresh liver is put in a plastic bag in the refrigerator, the blood will collect in the bag. Students may be able to bring some liver from home. A small amount of a blood (a drop or two) is enough for each test. Avoid frozen liver, if possible. Pretest the liver juice; if activity is too low, blend 10 grams of liver in 100 cm^3 of water for a few seconds and use this suspension as a source of catalase.

Range of Results Students should observe that both MnO_2 and liver juice function as catalysts. They should note that the activity of the inorganic catalyst is not destroyed by boiling, whereas enzyme activity is destroyed.

Postlab Discussion Compare results and discuss organic and inorganic catalysts. Inorganic catalysts, being crystalline, are not melted by such mild heat. Section *B-21 Breaking the Bonds* explains the effect heat has on protein.

Here are some questions you could use in a summary discussion of this experiment.

1. Do you have any evidence that MnO_2 catalyzes the breaking down of hydrogen peroxide instead of reacting with it?
2. Would you need additional steps in this procedure to confirm this? (Definite experimental evidence exists to show that manganese dioxide is indeed a catalyst in this reaction.)
3. Consider the formula of hydrogen peroxide and the kind of reaction you observed in test tube #2. What are the most likely products of the breakdown of hydrogen peroxide? How might you confirm your answer?
4. How do you explain the difference in activity between fresh liver juice and boiled liver juice?
5. Suppose that someone comparing test tube #2 and test tube #3 concluded that liver contained manganese dioxide. What evidence do you have either for or against this conclusion?

Miniexperiment Students can test for oxygen evolution in the preceding experiment by using the glowing splint test. A wide-mouth test tube should be used if this test is to be performed; otherwise, the foam may rise and extinguish the ember.

B-17 CHARACTERISTICS OF CATALYSTS

Experiment *B-16* is followed by material on reaction rate, active site, and enzyme nomenclature. An important concept is the high degree of specificity of enzyme-catalyzed reactions. Reaction rate can be defined either as the moles consumed per unit time or the moles per liter consumed per unit time. The choice is arbitrary, and so for simplicity we have chosen to use moles per unit time, eliminating the volume factor.

You may wish to show the CHEM Study film *Catalysis,* which contains a good discussion of inorganic catalysts as well as the enzyme peroxidase.

B-18 MOLECULAR ARCHITECTURE OF ENZYMES

This section begins to correlate enzyme structure and function. The first aspect of structure to be studied is the amino acid sequence of the protein. This structure results from amino acids becoming attached to each other through formation of peptide bonds. Peptide bond formation was covered in section *B-9 Proteins and Amino Acids.* Some proteins consist of several chains of amino acids held together by disulfide bridges and other forces (hydrogen bonds, etc.), which are discussed in the next section.

Determination of the sequence of amino acids in a protein that is hundreds of amino acids long is an extremely difficult task. Basically, what is done is to break the protein into a number of smaller pieces. The sequence of amino acids in

each piece is then determined by breaking off amino acids one at a time either by chemical reactions or by enzymatic degradation. The amino acids are then identified by chromatography. Later, the sequence of the whole protein can be determined by piecing together the smaller sequences.

ANSWERS TO EXERCISES

(Student module page 44)

1. $(2)^3 = 8$
2. $(3)^3 = 27$
3. $(20)^3 = 8000$

B-19 HOLDING THE FOLDING

Using the fact that proteins are folded in definite ways, we introduce four types of bonding that can explain this phenomenon. You can point out to students that the peptide bond has two roles:

1. It links amino acids in a specific sequence.
2. It helps keep the structure folded by forming hydrogen bonds with other peptide linkages or possibly other functional groups.

The side chains (*R* groups) are also important. They contain ammonium, carboxylate, hydroxyl, and other functional groups that form not only the disulfide bridges but also the ionic bonds and many of the hydrogen bonds in the protein. Even hydrophobic bonding can be attributed to the *R* groups. If the side chain is a hydrocarbon, that part of the molecule will be insoluble in water and will tend to interact with other nonpolar parts of the molecule. *Hydrophobic bond* is the term that is used to describe the apparent attraction of nonpolar side chains for each other.

The tendency of the nonpolar side chains to come together to form hydrophobic regions is one of the main factors stabilizing the folded structure of the protein. Students can review this material on hydrophobic bonds by going back to experiment *B-10 Solubilities of Biomolecules* and to section *B-11 Like Dissolves Like*. Students also may wish to review material on the ionic nature of amino acid side chains that was presented in sections *B-14 Amino Acids: Basic and Acidic Facts* and *B-15 Zwitterions: Negative and Positive*.

A word is in order about the relative magnitude of bond strengths. The bond strengths of the ionic, hydrogen, and hydrophobic bonds in proteins are roughly the same and are much weaker than covalent bonds. You may be surprised by this, since ionic bonds in crystals are usually about the same strength as covalent bonds. The ionic bonds in crystals are strong because the ionic species are tightly packed and not hydrated. In contrast, ionic species in proteins are not tightly packed and are hydrated, resulting in a weaker attraction between the opposite charges.

Many books and popular articles refer to the primary, secondary, tertiary, and quaternary structures of proteins. We will explain these terms because students may inquire about them. *Primary* simply refers to the sequence of amino acids in a peptide chain. *Secondary* refers specifically to hydrogen bonds formed between atoms of the peptide linkage itself. Those bonds are responsible for the α-helix and pleated-sheet structures. See a biochemistry reference for a discussion of these. *Tertiary* refers to the folding (in addition to the α-helix) held by the bonds and forces between the side chains (*R* groups).

The distinction between secondary and tertiary structures is artificial and does not serve to illuminate the principles of protein structures that we wish to emphasize. The disulfide bridges are usually considered part of the primary structure. We prefer to distinguish between sequence and folding.

Quaternary structure refers to assemblages of individual protein chains (i.e., protein subunits) held together by noncovalent bonds. Many enzymes consist of more than one protein chain. However, this information is not necessary for understanding enzymes at this time.

MINIEXPERIMENT

B-20 SUNNYSIDE UP OR POACHED

This experiment uses the ordinary act of cooking an egg to show the effect of excess heat and pH on the folding of a molecule. The purpose of the experiment is to show that heat and acid can denature a protein.

Concepts

- Proteins are denatured by extremes in pH and by exposure to excess heat.

- When a protein is denatured, its structural conformation is changed.
- Acid solutions and heat can each break the bonds that hold a protein in its specific conformation.
- When a protein is denatured, hydrogen bonds, ionic bonds, and hydrophobic bonds are broken. No covalent bonds need be broken to denature a protein.

Objectives

- Cook an egg with heat or acid.
- Explain that when a protein is denatured, its structural conformation is changed by the breaking of hydrogen, ionic, and hydrophobic bonds.

Estimated Time 20 minutes

Student Grouping Pairs

Materials

2 eggs, fresh (egg white—in small amount)
15 50-cm^3 beakers
15 150-cm^3 beakers
15 thermometers, −10°C to 110°C
15 ring stands and rings
15 wire gauze; asbestos centers
15 medicine droppers
15 Bunsen burners
15 cm^3 6 M HCl

Advance Preparation Have fresh eggs on hand or have students bring them. A few eggs will go a long way in this experiment.

Prelab Discussion Point out that egg white is a concentrated aqueous solution of protein.

Laboratory Tips In *Part A*, be sure students heat the water to at least 80°C so that coagulation occurs as soon as the egg white is added to the water. Some students may suggest trying to denature egg white with sodium hydroxide or another strong base; let them perform this operation to convince themselves. Unfortunately, the egg white will not precipitate in a strong base, even though it is denatured. It is a fortunate fact (for this experiment) that egg white is precipitated as it is denatured by hydrochloric acid. Although proteins, in general, are denatured at extreme pH values, they do not always precipitate.

Range of Results The egg white will coagulate and turn white in the hot water and acid.

Postlab Discussion You can point out that even though the egg white changed, it is still egg white. That is, only the secondary and tertiary structures of the protein were changed. Hydrogen, ionic, and hydrophobic bonds were broken but *the peptide bonds were not.* Breaking of these bonds and the resulting destruction of the primary structure can be accomplished by digestive enzymes and will be studied in section *B-30 Digestion: The First Step of Metabolism* and in miniexperiment *B-31 Enzymatic Digestion of Protein.*

B-21 BREAKING THE BONDS

Your lesson on this section can serve as a review of miniexperiment *B-20 Sunnyside Up or Poached.* You will be summarizing the behavior of proteins when they are heated (the behavior of egg white, for example, when it is heated). Stress that it is only the weak bonds which hold the protein in its folded position that are broken when the protein is subjected to high temperatures. Refer to the illustrations *Protein in Folded Position* and *Unfolded Protein* on page 51 of the student module.

ANSWERS TO EXERCISES

(Student module page 55)

1. **a.** Valine, leucine, isoleucine, and phenylalanine would be good choices.
   b. Lysine, as well as aspartic and glutamic acid would be good choices.
   c. cysteine
   d. serine, threonine, tyrosine, lysine, arginine, aspartic acid
2. It unfolds. Examples are many. Those included in this text are curling hair, cooking eggs, canning foods with sterilization, and pasteurization of milk.

B-22 pH: THE POWER OF HYDROGEN IONS

This section introduces the student to the concepts of pH and ionization of acids and bases. It is essential that the student have a firm grasp of these concepts before moving on to the next

three sections, *B-23*, *B-24*, and *B-25*, since these apply the concept of pH to the activity of an enzyme.

Supplementary material on pH, acids, and bases can be found in *Reactions and Reason: An Introductory Chemistry Module* and *Diversity and Periodicity: An Inorganic Chemistry Module*, as well as in any general chemistry textbook.

You may wish to give students some practice in the following:

1. Calculating the pH of a solution with a known hydrogen ion concentration.
2. Calculating the hydrogen and hydroxide ion concentrations of a solution with a known pH.
3. Writing a chemical reaction that illustrates the ionization of a strong acid in water.
4. Writing a chemical reaction that illustrates the ionization of a strong base in water.

ANSWERS TO EXERCISES

(Student module pages 56–57)

1. The pH of 0.1 M HCl is 1.
2. The H^+ concentration at pH 9 is 1×10^{-9} M.
3. The pH is equivalent to 10^{-1} M H^+, and pH 7 is equivalent to 10^{-7} M H^+. Therefore, $[H^+]$ at pH 1 is 10^6 times $[H^+]$ at pH 7.
4. The concentration of OH^- is 10^{-1} M. Therefore the pH is 13.
5. The concentration of HCl is 1 M. Therefore, the $[H^+]$ is 1 M and the pH is 0.
6. acidic (although not strongly)

ANSWERS TO EXERCISES

(Student module page 58)

1. 1.0×10^{-3} M
2. 1.0×10^{-1} M
3. 0.01 moles
4. 0.365 grams, or about 0.4 grams

MINIEXPERIMENT
B-23 INSPECTING THE EXPECTORATE

The purpose of this experiment is to illustrate that salivary amylase is active at the pH found in the mouth (neutral) and inactive at the pH found in the stomach (acid).

Concept

- Enzyme activity is dependent on pH.

Objective

- Cite experimental work that demonstrates that enzyme activity is dependent on pH.

Estimated Time 30 minutes

Student Grouping Pairs

Materials

60 18 × 150-mm test tubes
15 small dropper bottles of 2 M HCl
45 cm^3 of 1 percent starch
15 medicine droppers
30 pieces universal pH paper
15 small dropper bottles of starch solution
15 small dropper bottles of iodine solution

Advance Preparation The starch and iodine solutions are the same as those used in experiment *B-13 Chemical Reactions of Biomolecules*. Check the starch solution for mold if you use the same solution you prepared for experiment *B-13*. If possible, have burets set up for dispensing the starch and the iodine solutions.

Prelab Discussion None

Range of Results The amylase will work in the neutral pH range to break down the starch, and so after 10 minutes the iodine test in that test tube should be negative. Since the amylase is inactive in acid, the iodine test will be positive.

Postlab Discussion The results of miniexperiment *B-20 Sunnyside Up or Poached* can be tied in with this experiment, since both tests show that the structure of the protein is dependent upon the pH of the medium. Since an enzyme is a protein, its activity is a function of its structure (unfolded proteins do not work).

Miniexperiment To illustrate the effect of amylase on starch, students can chew a cracker, piece of bread, or piece of raw potato and note its taste. After a while, the student may notice that a sweet taste develops as the starch is converted into the disaccharide, maltose.

B-24 SUCCINATE DEHYDROGENASE

This section serves as a prelab discussion for the experiment that follows. Emphasize the calculation of reaction rates. Prepare your students to handle the data that the experiment provides.

Note: Plan to start experiment *B-39 Making Sauerkraut* soon. It takes about two weeks for the cabbage to ferment.

EXPERIMENT B-25 pH AND SUCCINATE DEHYDROGENASE

This experiment gives your students an opportunity to study the effect of small pH changes on enzyme activity. It also enables them to develop a quantitative concept of enzyme activity.

Concepts

- Enzyme activity can be expressed quantitatively in the same manner as other chemical reactions.
- Even small changes in pH can alter enzyme activity without necessarily destroying it.
- Changes in pH will disrupt weak bonding in a protein molecule—ionic, hydrogen, and hydrophobic bonds.

Objectives

- State that a change in pH will cause a change in enzyme activity.
- Calculate the rate of an enzymatic reaction when the data are given.
- Convert a rate from one metric unit to another.
- Use a pipet.
- Record experimental data accurately.

Estimated Time The experiment takes a full 40-to-50-minute class period. Many teachers have suggested that the students set up and add the buffer to their test tubes the day before the experiment is to be run. Others have their students actually prepare the buffers.

Student Grouping Pairs

Materials

75 18 × 150-mm test tubes
75 cm^3 buffer pH 6.3
75 cm^3 buffer pH 8.0
60 cm^3 0.1 M sodium succinate
60 cm^3 0.0003 M DPIP
15 disposable pipets, 1-cm^3
15 test-tube racks
150 cm^3 buffer pH 7.3
75 cm^3 buffer pH 12.0
60 cm^3 chicken heart homogenate (enzyme)
120 cm^3 mineral oil *or* vegetable oil
15 pieces graph paper, linear

Advance Preparation The buffers and other solutions will have to be prepared in advance. The pH 7.3 buffer will be used again in experiment *B-27 The Active Site* and miniexperiment *B-34 Making Light With ATP*. Preparing a little extra will save you time later on. The sodium succinate, DPIP, and enzyme homogenate are also used in experiment *B-27 The Active Site*. (If the buffer is refrigerated, it should stay good for the later experiments.)

1. *$Na_2HPO_4 \cdot 7H_2O$:* Dissolve 53.6 g of $Na_2HPO_4 \cdot 7H_2O$ in order to make 1 liter of solution. This is a 0.2 M solution.
2. *$NaH_2PO_4 \cdot H_2O$:* Dissolve 27.6 g of $NaH_2PO_4 \cdot H_2O$ in order to make 1 liter of solution. This is a 0.2 M solution.
3. *Buffer pH 6.3:* Add 20 cm^3 of No. 1 to 80 cm^3 of No. 2 and dilute to 200 cm^3.
4. *Buffer pH 7.3:* Add 75 cm^3 of No. 1 to 25 cm^3 of No. 2 and dilute to 200 cm^3.
5. *Buffer pH 8.0:* Add 94.7 cm^3 of No. 1 to 5.3 cm^3 of No. 2 and dilute to 200 cm^3.
6. *Buffer pH 12.0:* Add 27.0 cm^3 of 0.1 M NaOH to 125 cm^3 of solution No. 1, and then dilute to 200 cm^3.
7. *Sodium succinate (0.1 M):* Dissolve 2.7 g sodium succinate hexahydrate in 100 ml distilled water.
8. *DPIP (0.0003 M):* Dissolve 0.05 g of 2,6-dichlorophenolindophenol (sodium salt) in 500 cm^3 distilled water. 2,6-dichlorophenolindophenol is sold in 1.5- and 10-g bottles as "2,6-dichloroindophenol sodium salt" (Catalog No. 3463 by Eastman Organic Chemicals, Rochester, NY 14650).
9. *Enzyme homogenate:* To prepare, trim chicken hearts free of excess fat. Weigh the trimmed hearts and mince thoroughly. Place the minced tissue in a blender and add 100 cm^3 of pH 7.3 phosphate buffer for every 20 g of tissue. Five trimmed hearts weigh about 20 g.

Be sure the blender blades are covered by the fluid and blend for one minute at full speed. This should reduce the mixture to a relatively homogeneous and foamy suspension with no large pieces of heart remaining.

Add an additional 200 cm^3 distilled water for every 100 cm^3 buffer used in the first step, and mix with a stirring rod. This mixture can be centrifuged for about 10 minutes to remove large particles and debris, which will retard the rate of the enzyme reaction. Instead of centrifuging, you may also put the mixture in the refrigerator overnight and decant off the supernatant for use the next day.

The preparation will retain sufficient activity for classroom use for two days if refrigerated. You should test the activity of the preparation before you are ready to use it. A sample should react completely in 4 to 10 minutes. If the reaction is too fast, the enzyme can be diluted. If it is too slow, the amount of enzyme added by the students can be increased.

We have tried several different tissues for this experiment and have found that heart works best. We have recommended chicken heart only because it can be bought in small packages. Veal heart has also been found suitable. Liver and kidney are not suitable because competing reactions from compounds in the tissues interfere. Some teachers have found that chicken hearts can be obtained readily from kosher delicatessens. Otherwise, they are often packaged with liver and gizzards as giblets.

The enzyme must be allowed to stand for at least an hour before use so that the oxygen whipped up with the suspension can escape. If the oxygen remains, it keeps DPIP blue.

Note: If possible, use burets to dispense the buffers and dropper bottles for the enzyme. The DPIP should be added with a calibrated dropper or a pipet.

Prelab Discussion Remind students that since there is a quantitative aspect to this experiment, they will have to be accurate in measuring out the reactants. If pipets are used for any of the reagents, you may have to demonstrate how to use a rubber bulb to suck up the liquid, since it can not be done by mouth.

Some of the dye may bind to the protein, giving the solutions a slight blue color even after the reaction is completed. Thus, if students wait until all of the blue disappears before recording the time, they will have to wait a very long time. We suggest that you run the test as in tube #3, using a few tubes. This way the students will see what the endpoint looks like, and then they will be able to use the test tubes you prepared for comparison when they do the experiment.

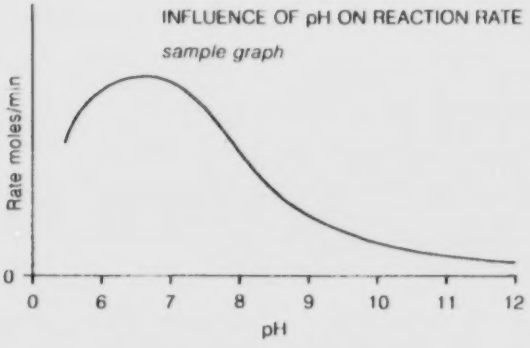
If you pretest this experiment the day before the experiment is run, you may find it will save time if the students prepare the tubes during the prelab so that when they are ready for the run it will be necessary only to add the enzyme solution and DPIP and time the reaction.

Point out that the procedure calls for adding a few drops of oil after the DPIP. This is a necessary step, since the oil covers the surface and keeps out oxygen that will prevent the DPIP from losing its blue color.

Postlab Discussion By pooling results, the class should come up with a fairly smooth curve that shows the large changes which result from small changes in pH. The optimum pH should be near the neutral point. As you raise the pH from 6 to 8, some weakly acidic groups in the protein will be deprotonated, resulting in a small change in charge in small regions of the protein. This can affect the binding of the negatively charged succinate molecule in the active site and also possibly cause small changes in the folding of the protein.

The succinate dehydrogenase reaction is an oxidation-reduction reaction in which the blue DPIP is decolorized as it is reduced.

The conversions involving exponents at the end of the experiment were included to demonstrate the convenience of using different units. They also provide students with practice in using exponents.



B-26 THE POTENT PART OF THE PROTEIN

In this section we begin to investigate the question of how an enzyme catalyzes a reaction. Some side chains of amino acids in the protein form ionic bonds and hydrogen bonds with a substrate molecule. These bonds "anchor" the substrate precisely in the active site of the enzyme. This is shown in the illustration, *Enzyme*, page 62 of

the student module. These bonds are similar to those that keep the folds of the protein in place. Remind the students that they have studied the formation and the breaking of these bonds in sections *B-19 Holding the Folding*, *B-20 Sunnyside Up or Poached*, and *B-21 Breaking the Bonds*. If necessary, review these sections with your class.

The CHEM Study film *Biochemistry and Molecular Structure* could be shown in conjunction with this section. It clearly depicts enzyme specificity. One section is devoted to the action of drugs as enzyme inhibitors. The anchor point idea is referred to as the lock-and-key mechanism in this film.

EXPERIMENT B-27 THE ACTIVE SITE

The purpose of this experiment is to give your students an opportunity to investigate the active site of an enzyme.

Concepts

- There exists a specific place on an enzyme molecule called the *active site*, where the reaction occurs.
- The active site will accept only molecules with appropriate specific structures and shapes.
- Substrates (reactants) are molecules that fit in the active site and will undergo reaction.
- Some molecules that fit in the active site but do not undergo reaction act as inhibitors.
- An inhibitor reduces the reaction rate by combining with the enzyme.

Objectives

- Predict whether other given molecules could also fit in the active site, given the structure of a substrate for some enzyme.
- Determine experimentally if a substrate analog is an inhibitor.

Estimated Time The experiment takes a full 40-to-50-minute class period. Many teachers have suggested that students set up and add buffer to their tubes on the day before the experiment.

Student Grouping Pairs

Materials

15 test-tube racks
525 cm^3 pH 7.3 buffer
45 cm^3 0.1 M sodium succinate
30 cm^3 0.2 M sodium propionate
90 cm^3 0.0003 M DPIP
90 18 × 150-cm test tubes
90 cm^3 enzyme homogenate
30 cm^3 0.2 M sodium malonate
15 disposable pipets, 1-cm^3
225 cm^3 of vegetable *or* mineral oil

Advance Preparation The phosphate buffer (pH 7.3), sodium succinate (0.1 M), DPIP (0.0003 M), and enzyme homogenate are prepared the same way they were in experiment *B-25*. In addition to these reagents you will need:

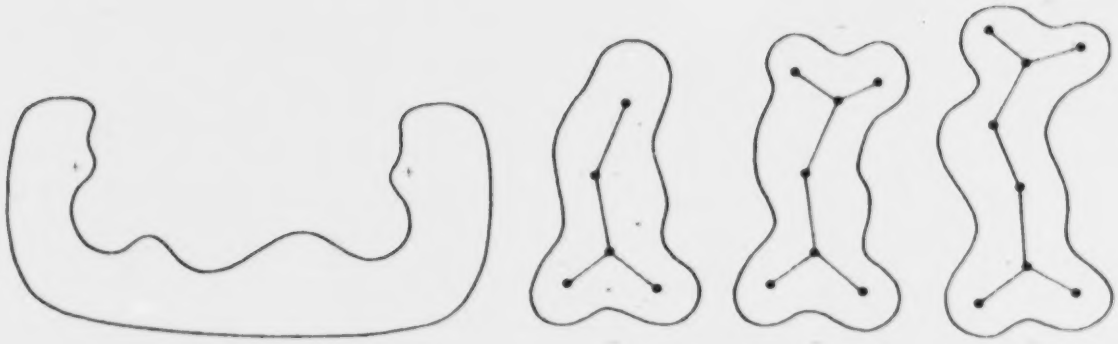
1. *Sodium malonate (0.2 M):* Dissolve 2.1 g malonic acid in about 50 cm^3 of distilled water and neutralize to a pH of between 6 and 7 (pH paper will do to check) with 2 M NaOH. Dilute the final solution to 100 cm^3 with distilled water.
2. *Sodium propionate (0.2 M):* Dissolve 1.5 g propionic acid in about 50 cm^3 of distilled water and neutralize as above. Bring the final solution to 100 cm^3 with distilled water.

Note: The pH 7.3 buffer will be needed again in *B-34*, so any remaining buffer should be stored in the refrigerator until then.

Prelab Discussion As in the previous experiment with DPIP, the reaction mixture must be covered with a few drops of oil to keep the oxygen out. The procedures are very much the same as in *B-25*. The emphasis is on discovering which analogs will bind to the active site. This is done by timing the reaction rate in the presence of analogs.

The CHEM Study film *Biochemistry and Molecular Structure*, which introduces the anchor point (lock-and-key) mechanism and the specificity of the active site, could be used for a prelab discussion to this experiment.

Postlab Discussion The questions at the end of the experiment will give a good basis for the postlab discussion, since they are aimed at bringing out what exactly happened in the experiment. We have tried to avoid the "lock-and-key" figures that are commonly used to indicate binding in the active site because most students can learn the chemical explanation with very little additional effort. The following diagrams may help to explain the results:



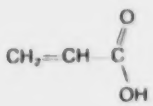
succinate dehydrogenase propionate malonate succinate

You can make cutouts similar to the ones shown above so that your students can see that the two substrate analogs as well as the substrate fit in the active site of the enzyme. They should recognize that only malonate and succinate will bind because of their charges. The propionate cannot bind adequately, since it has only one carboxyl group. Note that this model allows us to explain only binding and cannot be used to predict that an enzymatic reaction will occur. Thus an enzyme reaction can be viewed as a two-step process: (1) binding, and (2) the chemical conversion. For example, malonate can bind but does not react.

We must point out that *some* inhibitors do not interact directly with the active site. They combine with the enzyme at some distant position and change the folding of the enzyme.

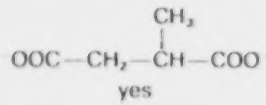
Answers to questions

1. No. Because there was no reaction in tubes E and F.
2. Yes. As shown by tube C (little or no reaction compared with tube B).
3. No. Because there is a normal reaction in tube D.
4. No. Two are needed. Malonate and succinate bind; propionate does not.
5. Any positively charged group. Students should be expected to answer "ammonium groups" ($R-NH_3^+$). There must be two, one for each carboxyl group.
6. Students should answer yes. The product would be propenoic acid (acrylic acid).

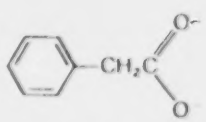


7. There is no way to form a double bond in malonate. If the enzyme removed two hydrogens from malonate, the product would have to have five bonds around one of the carbon atoms.

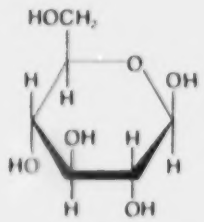
Miniexperiment If sufficient material is available, have the students build a model of the substrate and some analogs that might bind in the active site. Then have them try to fit the models into a previously built "model" of the active site. Ask students to identify which molecules will be potent inhibitors of succinate dehydrogenase. Models of the following compounds would be suitable for this purpose.



yes



no



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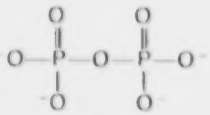

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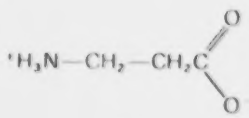

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B-28 OTHER FEATURES OF THE ACTIVE SITE

This section describes some of the other features of the active site. Emphasize that substances other than amino acids are intimately involved in enzyme functions. One obvious example is hemoglobin, which contains iron. Material in this section can be related to previous material found in *Diversity and Periodicity: An Inorganic Chemistry Module*, as well as to the current concern with lead and mercury poisoning.

Note: If you haven't already done so, start *B-39 Making Sauerkraut* now!

EXPERIMENT
B-29 TEMPERATURE AND REACTION RATES

The purpose of this experiment is to demonstrate the effect of temperature on the rate of an enzymatic reaction.

Concept

- Enzyme-catalyzed reactions are very sensitive to temperature changes.

Objective

- Predict the effect of temperature on an enzymatic reaction, within limits.

Estimated Time 45 minutes

Student Grouping Pairs

Materials

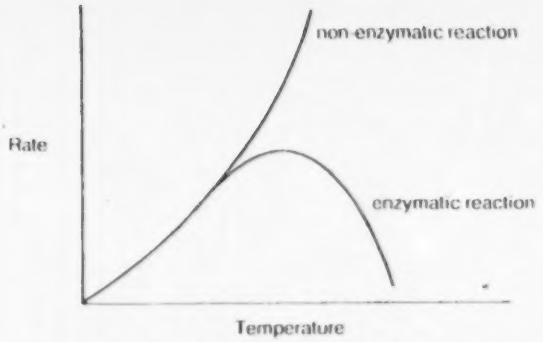
15 18 × 150-mm test tubes
15 test-tube racks
45 100-cm³ beakers
30 250-cm³ beakers
15 1-cm³ disposable pipets
15 stirring rods
15 pieces graph paper, linear
hot water (45°C)
15 rennet tablets
2 liters milk (fresh skim milk preferred)
15 thermometers, −10°C to 110°C

Advance Preparation One quart of milk will be enough for seven groups of students. Whole milk can be substituted for skim milk, but the initial curd formation is easier to see with skim milk. Nonfat dry milk can also be used, but some teachers have found that curd formation is slow unless a more concentrated solution than described on the package is prepared. The experiments have been successful if the milk is reconstituted with only two-thirds the amount of water called for in the recipe. This may vary from batch to batch. We suggest you pretest your dry milk before using it in class. Sodium caseinate is available from some suppliers, but we do not recommend it, since curd formation will not occur unless calcium salts are added. The rennet suspension may be prepared in advance, if desired. It has been found to keep for four days. Hot tap water can be used in the 45°C water bath.

Prelab Discussion Students should be reminded to heat only the milk and not the rennet suspension. (Unless stated carefully, this reminder may give away the answer to question 1.) Students should recall their experience with the effect of high temperature on enzyme activity and protein structure (*B-16* and *B-20*) and may be asked to predict what the effect of low temperature will be on an enzymatic reaction. Students should also be reminded to clean the stirring rod to avoid transferring curds or enzyme from one sample to another.

Postlab Discussion The results will show that increased temperature results in an increased reaction rate. However, this fact must be tied in with the other fact that students have observed—namely, that proteins are denatured by high temperatures. In this respect, enzymatic reactions differ from other chemical reactions, and it is to be hoped that students will be able to tie both ideas together and conclude that enzymes will have a maximum temperature for reacting. Lower temperatures will slow the reaction rate, and higher temperatures will eventually denature the enzyme, thus stopping the reaction.

You may ask students to predict what the maximum temperature might be. Possibly they will suggest a body temperature that is slightly below the maximum temperature for most enzymes found in human beings. The maximum is variable and depends greatly on the enzyme under discussion.



Temperature dependence of rate of typical enzymatic and nonenzymatic reactions

Answers to questions

1. In order to show that the results were due to the enzymatic activity of the rennet preparation, the experiment can be repeated, this time substituting distilled water for the rennet preparation. Also the rennet could be boiled, thereby destroying the enzyme activity.
2. Test with the Biuret reagent (See experiment *B-13*)
3. Cooling the milk would slow down the appearance of curds.

The protein precipitated in this experiment is present in native milk as a very large colloidal protein complex called the casein micelle. The protein in the micelle is quite heterogeneous, consisting primarily of 45–55 percent Alpha-caseins, 25–35 percent Beta-caseins, and 8–15 percent Kappa-caseins. The designations Alpha-, Beta-, and Kappa- refer to the electrophoretic behavior of these three classes of caseins. The casein micelles range in size from 50–250 μm and cause considerable light scattering, resulting in the "milky" appearance of skimmed milk. At the pH and salt concentrations present in native milk, these micelles are quite stable over a large temperature range; however, relatively minor alterations in the casein micelle itself or in the ionic environment of the micelle can destabilize the micellar suspension and result in clotting.

The clotting phenomenon observed in the experiment is a complex process consisting of an enzymatic and nonenzymatic phase. In the first phase, the enzyme cleaves a specific peptide linkage in Kappa-casein resulting in an altered casein micelle. In the nonenzymatic step the altered micelles aggregate to form the curd. The two steps overlap and both are temperature dependent. For the first phase, as with any enzymatic reaction, there will be an increase in reaction rate with increasing temperature up to the temperature at which the enzyme is denatured. Superimposed upon this is the temperature effect upon the nonenzymatic phase. The latter is not completely understood and is markedly affected by the temperature history of the milk. If the milk is aged at temperatures below 8°C and enzyme is added, although the enzymatic reaction proceeds at a reduced rate, little or no clotting is observed. But upon subsequent heating an immediate and spectacular curd formation results. Thus milk stored at refrigerator temperatures and heated to 45°C prior to the addition of enzyme would give different results than if enzyme was added to stored refrigerated milk prior to the heating step.

The rennet preparation used in this experiment is derived from an extract of calf's stomach. The extract is precipitated by the addition of acid or sodium chloride. Gelatin is often added to assist in the complete precipitation and recovery of the rennin from the extract. Rennin is not the only protease that can be used to clot milk. Many plant, animal, and microbial proteases, including pepsin and papain have been investigated and shown to clot milk; however, most are extremely proteolytic, causing extensive degradation of the casein curd, and resulting in poor yields and in flavors and properties unsuitable for use in making cheeses.

EVALUATION ITEMS

These are additional evaluation items that you may wish to use with your students at various times during the preceding section. The correct answer to each question is indicated by shading.

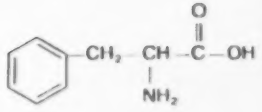
1. The following data were gathered when two catalysts, A and B, were added to hydrogen peroxide:

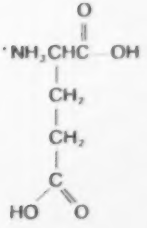
   A gave a reaction.
   A + heat gave a reaction.
   A + H^+ gave a reaction.
   B gave a reaction.
   B + heat gave no reaction.
   B + H^+ gave no reaction.

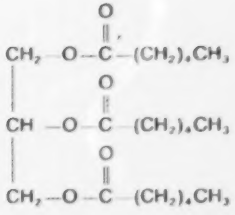
   On the basis of the above information, which of the following is true?

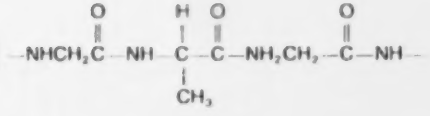
A. A and B were both enzymes.
B. A and B were both inorganic catalysts.
C. A was an enzyme, B was an inorganic catalyst.
D. A was an inorganic catalyst, B was an enzyme.

2. Which of the following structures has a peptide linkage?

A. 

B. 

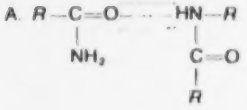
C. 

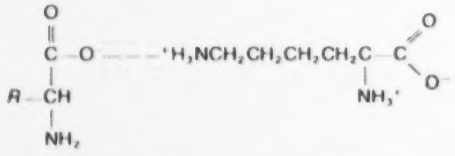
D. 

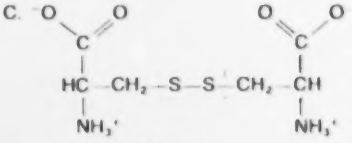
3. Two enzymes contain the same kind and number of amino acids. What, then, makes one different from the other?

The enzymes differ in the sequence of amino acids in the protein chain.

4. For each structure below, indicate the type of bond illustrated by the shaded area.

A. 

B. 

C. 

A. hydrogen bond

B. ionic bond

C. disulfide bridge

5. Name the types of bonds described below.

A. A disulfide bridge is formed between thiol groups.

B. An ionic bond is the result of the attraction of positively and negatively charged groups.

C. A hydrophobic bond results from side chains that do not contain functional groups.

6. A hydrogen bond is similar to a very weak ionic bond. T or F

7. Adding acid to a protein affects which type of bonds? How does it affect them?

Acid destroys ionic bonds between carboxylate and amino groups by protonating the carboxylate and removing its negative charge.

8. Give two examples of household activities that illustrate protein denaturing.

pickling and cooking

9. What is the hydrogen ion concentration of a solution that has a pH of 5? 10^{-5} M

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10. What is the pH of a solution that has a pOH of 4? [10]

11. What is the pH of a 0.01 M solution of HCl? [2]

12. How many moles of HCl are there in 100 cm^3 of stomach juice that has a pH of 2? [0.001]

13. What evidence is there that the enzyme amylase works in the mouth but not in the stomach?

[Amylase was inactive when acid was added to the reaction mixture in miniexperiment *B-23*. The stomach is also very acidic.]

14. Consider the following data for enzyme X:

pH	*Moles consumed*	*Time (sec.)*
5	1.5	45
7	1.7	70
3	0.5	30
9	0.7	60
1	0.8	80

What is the optimum pH range for the enzyme?

A. 6.5–7.5
C. 3.5–4.5
[B.] 4.5–5.5
D. 5.5–6.5

15. If 2 cm^3 of 0.005 M DPIP turns colorless in 2.5 minutes, the reaction rate is:

[A.] 4 μmoles per minute
B. 40 μmoles per minute
C. 50 μmoles per minute
D. 80 μmoles per minute

16. $^-OOC-(CH_2)_5-NH_3^+$ is a substrate for Enzyme E. Another possible substrate might be:

A. $^-OOC-(CH_2)_2-CH_3$
[B.] $^+H_3N-(CH_2)_4-COO^-$
C. $^-OOC-(CH_2)_5-COO^-$
D. $^+H_3N-(CH_2)_5-C_6H_6$

17. An enzyme inhibitor will often be:

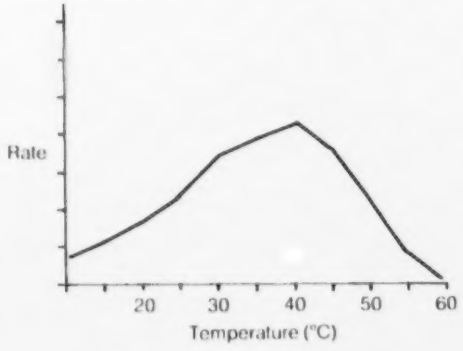
A. an enzyme
[C.] a substrate analog
B. a catalyst
D. a dehydrogenase

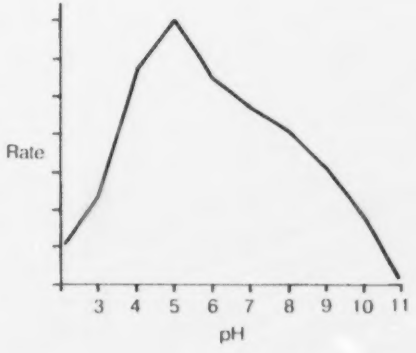
18. Give one reason why the mercury(II) ion is a poison.

[Mercury(II) can combine with the sulfhydryl groups in the active sites of enzymes, thereby inactivating them.]

19. If the data in the graphs below were obtained with the same enzyme, under what conditions would the reaction be fastest?

A. 25°C and pH 7
B. 50°C and pH 7
[C.] 40°C and pH 5
D. 45°C and pH 8
E. 40°C and pH 7





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Metabolism: The Community of Enzyme Reactions

This part of the module uses the student's understanding of enzymes and proteins to focus attention on how processes such as metabolism proceed within our bodies. Metabolism is considered in its broadest sense and digestion is used to introduce the process. Subsequently, metabolic pathways within cells are discussed. The necessity of energy to run physiological processes is treated, and there are sections on the Krebs cycle and the respiratory chain. The two final sections expand the notion of metabolism to explain the phenomenon of fermentation.

Basically, metabolism deals with two types of reactions. One type includes reactions that break down molecules, such as carbohydrates, to produce energy. The other type includes reactions that synthesize molecules such as proteins, lipids, and carbohydrates.

The word *metabolism* is derived from Greek and means "change." It is defined as the chemical changes in living cells by which energy is provided for the vital processes and activities and new material is assimilated to repair cells and build new ones. Sometimes the terms *anabolism* (Gr., "rebuild"), referring to constructive metabolism and *catabolism* (Gr., "throwing down"), referring to destructive metabolism, are used to describe the two phases of metabolism.

B-30 DIGESTION: THE FIRST STEP OF METABOLISM

The major point in this section is that the digestive enzymes secreted by the body all catalyze the same type of chemical reaction—hydrolysis. Earlier in this module, we showed that many big molecules, especially macromolecules, are formed by condensations in which water is released as a product *(B-7, B-8, B-9)*. Now, in order to reverse the process, water must be a reactant, and the reverse reaction is called hydrolysis. This relationship between condensation and hydrolysis is a good example of the reversibility of chemical reactions.

MINIEXPERIMENT
B-31 ENZYMATIC DIGESTION OF PROTEIN

The purpose of this miniexperiment is to demonstrate the enzymatic hydrolysis of a protein.

Concept

- The enzymatic breakdown of a protein is the hydrolysis of the peptide bonds to form small peptides and/or amino acids.

Objective

- Explain what meat tenderizer does, in biochemical terms.

Estimated Time 20 to 30 minutes (Students will spend much of this time waiting for the reaction to occur; they can be involved in other activities at the same time.)

Student Grouping Individuals

Materials

75 cm^3 10 percent gelatin solution
15 stirring rods
15 50-cm^3 beakers
7.5 g meat tenderizer
ice

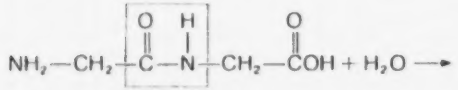
Advance Preparation A 10 percent gelatin solution is prepared by dissolving 10 g gelatin in 90 cm^3 hot water. Stir and continue to heat until the solution is complete. Then let the solution cool to room temperature. You may purchase meat tenderizer at any local food store or ask students to bring a small amount of it from home. If anyone uses monosodium glutamate (MSG) in the belief it is a meat tenderizer, he or she will find out otherwise in this experiment. MSG is actually only a flavor enhancer.

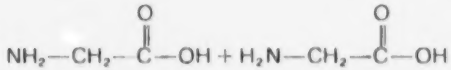
Prelab Discussion At first glance it may seem that this experiment is out of place—that it should have appeared in the unit on enzymes. Its purpose here is to help students correlate enzyme activity with metabolic processes such as digestion. Besides pointing this out, it might be good to review the basic composition of proteins so students can relate the enzyme activity of digestion with the breaking of peptide bonds by hydrolysis.

You may need to explain that gelatin forms a gel (for more on gels, see *Communities of Molecules: A Physical Chemistry Module*). The long protein strands form a lattice structure throughout the water phase. The strands of the protein molecule are attracted to each other by the same type of bonds that hold other proteins in their folded structures.

Range of Results The addition of the meat tenderizer to the solidified gelatin will cause digestion of the gelatin. The students will observe that the rubbery solid liquefies.

Postlab Discussion The gelatin liquefies because the long protein strands are "clipped" into short pieces by the enzyme. Point out that digestion is a hydrolysis reaction. The addition of a molecule of water to the peptide bond may be illustrated as follows:





Answers to questions

Do you think you could make a Jell-O salad with fresh pineapple? No, Jell-O is made from gelatin, and in the presence of the enzyme bromelian (similar to papain) in fresh pineapple, it would never gel. Do you think you could make a Jell-O salad with cooked or canned pineapple? Why? Yes, because the enzymes in pineapple are denatured by the extreme heat used in canning and cooking the pineapple.

B-32 THE COMPONENTS OF METABOLISM

The oxidation of glucose to carbon dioxide and water is used as an example of metabolism that occurs inside a cell. Some new terms such as *metabolic pathway, metabolite, cofactor,* and *vitamin* are introduced. (In the text, vitamins are introduced as parts of cofactors. The students should not be allowed to conclude that all vitamins are incorporated into cofactors. The biochemistry of some vitamins is far from completely understood.)

It is important that students realize that the reactions associated with each metabolic pathway proceed in a specific order. A major concept of this section is that metabolism is a highly ordered activity that can be broken down into discrete portions for analysis. The emphasis should be placed on the general concept of a metabolic pathway and the basic elements found in it. While the students should understand the general nature of these reactions, they should not memorize the pathway in detail. We have divided glucose metabolism into three major sections: glycolysis, Krebs cycle, and respiratory chain. These are developed in the next few sections of the module.

Note that we have used $NADH_2$ to represent the reduced form of NAD. This is a simplification done for the benefit of the students. The actual reaction, as it takes place in cells, is shown below:

$$XH_2 + NAD^+ \longrightarrow X + NADH + H^+$$

In the case of FAD, we have used the accepted abbrevations.

B-33 GLYCOLYSIS: A METABOLIC PATHWAY

We continue the treatment of metabolism that was introduced in the previous section by taking a closer look at a part of glucose metabolism, glycolysis. If students are reminded often of the general picture of what is going on, they will not get lost in detail or drown in what might seem like a bowl of alphabet soup.

An important compound in energy metabolism in living tissue is adenosine triphosphate (ATP). The bonds in such compounds release large amounts of energy when hydrolyzed. Thus, these compounds are said to contain high-energy bonds. Since only limited amounts of ATP are available at any one time, there must be ways to generate this substance. In the oxidative metabolism of glucose, about 40 percent of the 686 000 calories theoretically available from oxidizing one mole of glucose is conserved by the formation of the high-energy phosphate compound ATP. Most of the ATP is formed in the respiratory chain rather than by glycolysis.

If some students try to balance the overall glycolysis equation, there are some things they

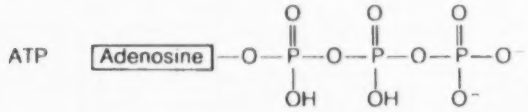
will have to keep in mind about dissociation of the acids in metabolism.

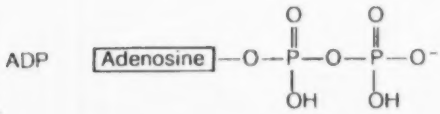
The overall reaction is:

$$C_6H_{12}O_6 + 2 \text{ phosphate} + 2 \text{ ADP} + 2 \text{ NAD} \longrightarrow 2C_3H_4O_3 + 2 \text{ ATP} + 2 NADH_2 + 2 \text{ water}$$

(glucose) (pyruvic acid)

Remember that in the cell pyruvic acid is normally dissociated into pyruvate and H^+. For the purposes of this reaction, phosphate and ADP are assumed to bear a single negative charge and ATP a double negative charge.





Phosphate $H_2PO_4^-$

The water can be thought of as coming from the synthesis of ATP from ADP and phosphate:

$$ADP + H_2PO_4^- \longrightarrow ATP + H_2O$$

EXPERIMENT
B-34 MAKING LIGHT WITH ATP

The purpose of this experiment is to show that ATP is the chemical that provides the energy needed to light a firefly.

Concept

- ATP provides energy for metabolic reactions.

Objectives

- Extract an enzyme and a substrate from a piece of tissue.
- Explain the rule of ATP in the reaction.

Estimated Time 30 minutes

Student Grouping If you purchase your fireflies, students can work in groups, or this can be a class demonstration. If students catch their own fireflies, they should work individually.

Materials

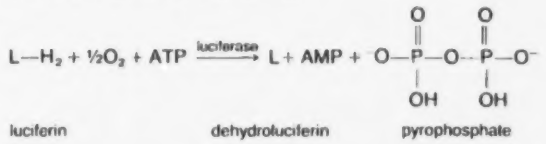
15 g sand (clean)
fireflies, approx. 150
15 mortars and pestles
15 18 × 150-mm test tubes
15 medicine droppers
15 50-cm^3 dropper bottles ATP solution
45 cm^3 phosphate buffer, pH 7.3
300 mg $MgSO_4 \cdot 7 H_2O$
shoe box for viewing chamber (optional)

Note: If you want to store ATP from one year to the next, it should be in a tightly capped bottle placed in a larger bottle containing desiccant. Store it in a freezer.

Advance Preparation Obtain firefly tails. If students catch their fireflies, be sure to use at least 10 fireflies per group. If you purchase fireflies, plan on using 50 mg dissected tails per demonstration. Test before using. To prepare the ATP solution, dissolve 50 mg ATP in 100 cm^3 distilled water. Note that ATP is easily hydrolyzed and should be prepared fresh daily. The pH 7.3 phosphate buffer that you used in experiment *B-25 pH and Succinate Dehydrogenase* should be satisfactory for this experiment if it has been kept refrigerated.

Set out the solid $MgSO_4 \cdot 7H_2O$ and sand. You may need to provide a shoe box with a hole in it to view the glowing solution if no dark room is available.

Prelab Discussion The luciferin reaction is different from most of the reactions we have discussed so far. The reaction is shown below:



Note that ATP is hydrolyzed to AMP and pyrophosphate. ATP has two high-energy phosphate bonds. In the reaction above, the last two phosphate residues are released together.

Many other organisms exhibit bioluminescence. Examples are clams, bacteria, worms, and jellyfish. Students may be familiar with the common fungus that glows (at night) in the woods. The biological importance of

bioluminescence is not known for many organisms. However, in fireflies, it is believed that the light serves to attract the opposite sex.

Not all bioluminescent reactions involve ATP. A good example is the substance aequorin, which is a luminescent protein found in a species of jellyfish. Some investigators believe that the energy for the light reaction in this instance comes from a conformational change in the protein structure.

Laboratory Tips Students must grind the firefly tails vigorously for at least five minutes to extract the enzyme. Be sure to kill the fireflies by freezing or with ether several minutes before use to allow the endogenous ATP to be hydrolyzed by the enzymes in the tail. If this is not done, the substrate luciferin will be used up during the grinding process and no light will be produced when ATP is added by the student.

Range of Results There is an immediate production of light after adding ATP.

Postlab Discussion You might wish to relate this reaction to the luminol reaction discussed in *Form and Function: An Organic Chemistry Module*, experiment *O-53 Chemiluminescence of Luminol.*

Note: Those who wish to demonstrate the effect of ATP on muscle contraction may purchase kits containing glycerinated muscle from Carolina Biological Supply Company. Firefly kits may be obtained from most biochemical supply houses, including: Carolina Biological Supply Company (Burlington, NC 27215); Calbiochem-Behring-Corp. (10933 North Torrey Pines Road, La Jolla, CA 92037); and Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178).

B-35 THE KREBS CYCLE

B-36 THE RESPIRATORY CHAIN

These sections complete our study of glucose metabolism. Again, you may have to keep students from becoming lost in details. One way to keep the general picture before the class is to use a large diagram or an overhead transparency as the basis for discussion. Pointing out to students where *they* are in the chain will help. Although we do not want to emphasize the complexity, we do not want to ignore it either. The Krebs cycle is also known as the *citric acid cycle* and the *tricarboxylic acid cycle*. It is named after the great biochemist, Sir Hans Krebs, who had much to do with its elucidation.

Be sure to emphasize that most of the ATP derived from glucose metabolism is produced in the respiratory chain. Some students may have heard of the electron transport chain. This is another name given to the respiratory chain, since electrons rather than H atoms are actually transferred from the reduced cofactors to oxygen. We have deliberately avoided discussion of the details of the electron transport reactions in the student module, since we felt it was inappropriate for students at this level. The intricate details of the electron transport chain are thoroughly discussed in any college-level biochemistry text.

B-37 BRANCHING

In order to see glucose metabolism in a wider context, the concept of branching is introduced here. This section ties together many things that have been covered in the module so far, and it is hoped that students will feel some sense of accomplishment at being able to talk about proteins, lipids, ATP, etc. As in the previous sections, keeping a diagram or projection before the class will help to keep the material in a general frame of reference. Be sure the students realize that many enzymatic steps are involved in the other pathways before fats and proteins can enter glycolysis or the Krebs cycle.

B-38 THE VERSATILITY OF METABOLISM

Fermentation is the unifying theme of this section. It is used to relate such dissimilar things as beer, penicillin, and yogurt. You may wish to have students do some research on some of the products of fermentation, including antibiotics, and report their findings to the class. Presentations of this type will prove useful in providing background for the next section (concerning the fermentation of cabbage to make sauerkraut). Ask students to bring sauerkraut recipes from home to compare with the one presented in the experiment *B-39*.

EXPERIMENT
B-39 MAKING SAUERKRAUT

The purpose of this experiment is to illustrate the metabolic process of fermentation and to provide experience in titration.

Concepts

- Sugars in cabbage are metabolized by anerobic bacteria (bacteria that do not require oxygen) and converted to lactic acid.
- The amount of lactic acid produced can be measured by titration with a base.

Objectives

- Determine the pH of a solution, using universal indicator paper.
- Determine the acid content of a solution by titration with standard sodium hydroxide.
- Relate the production of lactic acid to metabolism.

Estimated Time Day 1: 25 minutes—preparation and discussion of sauerkraut. Intervening days, 2 through 8: take a minute every other day to measure the pH of the fermenting solution with pH paper. Be sure the cabbage remains covered with water. Final day: one class period—decantation of sauerkraut juice and titration.

Student Grouping Pairs

Materials

15 120-cm^3 (4-oz.) wide-mouth jars with screw cap
1 to 3 graters, cabbage
600 cm^3 0.05 M NaOH
15 250-cm^3 Erlenmeyer flasks
1 dropper bottle phenolphthalein indicator
15 sets pH paper, universal
3 fresh cabbages
15 100-cm^3 beakers
18 g sodium chloride (1.2 g per student group)
15 burets with stands

Prelab Discussion Depending on which modules have been covered, it may be necessary to review titration techniques. Students should be cautioned to sprinkle the salt evenly over the shredded cabbage and not to use too much salt, since it will kill the bacteria. The cabbage should be kept completely covered with water for the two-week period. Someone will have to be in charge of replacing evaporated water.

Range of Results The surface of the sauerkraut may become moldy during the experiment, but this does not ruin anything, since juice from the bottom of the jar can be used for the titration. The pH of the juice should be approximately 3, but the concentration of the acid should be approximately 0.1 M. This would result in a pH of 1 if the juice contained strong acids (HCl). Thus, the results of this experiment provide a convenient means of introducing the concept of strong and weak acids. Lactic acid is a weak acid with a pH of 3.86. (At a pH of 3.86, half the lactic acid molecules will have been neutralized by OH^- ions.)

Postlab Discussion Compare results and calculations. The metabolic pathway may be briefly illustrated as:

saccharides in cabbage ⟶ glucose ⟶
glucose-6-phosphate ⟶
glyceraldehyde-3-phosphate ⟶ pyruvate ⟶
lactic acid (excreted by anerobic bacteria).

Note: Lactic acid is not the only acid produced. Other acids, notably acetic acid, are also produced during this fermentation. We have avoided discussing this in the student module to simplify the metabolic picture.

ANSWERS TO EXERCISES

(Student module page 89)

1. The reaction is called hydrolysis and takes place in the digestive tract.
2. The three pathways of glucose metabolism discussed in this module are glycolysis, the Krebs cycle, and the respiratory chain.
3. **a.** The enzyme is phosphofructokinase.
   b. The two phosphorylated sugars are metabolites. Although students have not been taught the names, they should know which compounds are metabolites. The compounds are fructose-6-phosphate and fructose-1,6-diphosphate.
   c. ATP and ADP are examples of cofactors.

4. a. the Krebs cycle
   b. the respiratory chain
   c. the Krebs cycle
   d. the respiratory chain
   e. the Krebs cycle
   f. glycolysis
   g. the Krebs cycle
   h. the Krebs cycle
   i. the respiratory chain
5. The pyruvic acid resulting from the glycolysis pathway in the yeast is converted to ethanol and carbon dioxide. It is the gaseous CO_2 that causes bread to rise.

EVALUATION ITEMS

These are additional evaluation items that you may wish to use with your students at various times during the preceding section. The correct answer to each question is indicated by shading.

1. Chemically speaking, digestion is basically:

   A. dehydrogenation
   [B.] hydrolysis
   C. hydrogenation
   D. anabolism

2. Which enzyme hydrolyzes triglycerides to fatty acids and glycerol?

   [A.] lipase
   B. amylase
   C. maltase
   D. pepsin
   E. trypsin

3. Ribonuclease would catalyze a reaction on which of the following?

   A. DNA [B.] RNA C. ribose D. none of these

4. The main function of the cofactor ATP in metabolism is

   A. to restore ionic balance at both sides of the cellular membrane.
   B. to catalyze the oxidation of the metabolites produced in the Krebs cycle.
   [C.] to transfer energy from one reaction to another.
   D. to synthesize amino acids.

5. A cofactor that transfers hydrogen atoms in metabolism is:

   A. ATP B. ADP [C.] $NADH_2$ D. pyruvate

6. Most of the ATP formed in metabolism is made in

   A. the beginning digestion reactions.
   B. the glycolysis pathway.
   C. the Krebs cycle.
   [D.] the respiratory chain.

7. In metabolism, the vitamin riboflavin forms a part of the cofactor:

   [A.] FAD
   B. ATP
   C. NAD
   D. citrate
   E. none of these

8. Briefly state how a triglyceride (a lipid) can be used to produce metabolic energy.

   Triglycerides can be converted to acetate which then enters the Krebs cycle where it is converted to CO_2 and water. During this process, it produces $NADH_2$ and $FADH_2$ which are used in the respiratory chain to produce ATP.

9. Identify the metabolic pathways that glycerol and fatty acids enter when metabolized to produce ATP.

   Glycerol enters glycolysis. Fatty acids are converted to acetate which enters the Krebs cycle.

The Organization of Cellular Activities

The cell is the theme of this final section of the module. The students will first encounter an overview of the cell and its parts. Then each of the important parts is treated, and its basic properties are discussed. The final sections focus on the nucleus and the fundamental activities performed by the DNA and RNA molecules.

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B-40 ORGANELLES: LITTLE ORGANS IN CELLS

This section serves to introduce the cell and some of its parts. It is suggested that there is a connection between the chemical activities that have been studied so far and the parts of the cell. Research in this area is ongoing, and much remains to be understood about the cell's parts and their functions. The terms *organelles* and *subcellular particles* are used interchangeably.

B-41 CELL MEMBRANE: GATEWAY TO THE CELL

The student is introduced to the concept that cell membranes are "active" in allowing specific molecules either to enter or leave the cell.

Active transport is briefly mentioned. Point out that this metabolic process requires the cell to expend energy. Molecules can also enter or leave a cell by another process, called *diffusion*. This process is a function of the structural characteristics of the membrane and the molecule, as well as the concentration of the molecules on each side of the membrane—that is, inside and outside the cell. The diffusion process is illustrated in the following experiment.

The composition of cell membranes varies, but they are basically composed of lipids and proteins. Although the exact arrangement of the lipids and proteins is a matter of controversy, it is thought that membranes contain 35 to 50 percent lipids.

As a general rule, if a molecule is highly polar (water is an exception), it can probably enter the cell efficiently only by a transport mechanism. However, if a molecule has appreciable lipid solubility, it can usually enter the cell by diffusion. It simply dissolves in the lipid of the membrane and diffuses to the other side. Highly polar molecules can diffuse through dialysis tubing, since it is made of regenerated cellulose and has relatively large water-filled pores through which molecules can diffuse. Of course, dialysis tubing cannot carry on active transport.

EXPERIMENT B-42 ARTIFICIAL MEMBRANES

The purpose of this experiment is to identify experimentally those biomolecules that diffuse through a semipermeable membrane.

Concepts

- Some molecules will diffuse through a membrane; some will not.
- Dialysis technique can be used to physically separate small and large molecules.
- Membranes are semipermeable barriers.

Objectives

- Use known chemical tests to identify biomolecules that pass through dialysis tubing.
- Predict whether a substance familiar to the students will pass through a dialysis membrane (based on the molecular size).

Estimated Time One period—10 minutes to initiate dialysis; 20-minute discussion while dialysis proceeds (during this time chemical tests can be reviewed; refer to experiment *B-13 Chemical Reactions of Biomolecules*); and then 15 minutes to test what came through the bag.

Student Grouping Pairs

Materials

180-cm strip of dialysis tubing
15 150-cm^3 beakers
90 cm^3 1 percent starch
90 cm^3 1 percent gelatin
90 cm^3 1 percent glucose
90 cm^3 1 percent monosodium glutamate
15 cm^3 iodine reagent
30 cm^3 Benedict's reagent
30 cm^3 ninhydrin reagent
30 cm^3 10 percent pyridine solution
30 cm^3 Biuret reagent

Advance Preparation Precut dialysis tubing (12-cm strip per group), and prepare solutions if you do not have enough remaining from experiment *B-13 Chemical Reactions of Biomolecules*. The reagents used here are the same as those used in experiment *B-13*.

Prelab Discussion Students who have studied *Communities of Molecules: A Physical Chemistry Module* will already have used dialysis tubing and may remember how it works. It would be best to forgo any explanation of how dialysis tubing works so that the students can figure it out from the experimental results. Demonstrate the technique of filling the dialysis tubing with solution, and then sealing it with either an overhand knot

or by tying the ends with string. *Caution students not to blow into the tube to open it.* They should be able to explain what would happen to the starch if the amylase in saliva got into the tube.

Range of Results The results are indicated in the following table.

	Iodine	*Ninhydrin*	*Benedict's Solution*	*Biuret*
Starch	+	–	–	–
Gelatin	–	+	–	+
Glucose	–	–	+	–
Monosodium glutamate	–	+	–	–

Postlab Discussion Students should have no trouble concluding which biomolecules came through the dialysis tubing, and more than likely they will be able to conclude that the tube separated biomolecules on the basis of size. You might also be able to get students to conclude that the diffusion process does not require the expenditure of energy. The questions in the experiment will also provide material for discussion.

Answers to questions

1. See notes for preceding section *(B-41)*.
2. The following would remain in the cell: succinate dehydrogenase, and catalase.
3. See notes for preceding section *(B-41)*.

B-43 ARTIFICIAL KIDNEY MACHINE

This section has been included to illustrate that the basic principle of the previous experiment has important medical applications.

B-44 MITOCHONDRIA: POWERPLANTS IN CELLS

Point out that mitochondria can be found in high concentration in organs that use a great deal of energy, such as the heart and the brain, whereas mitochondria are found in low concentration in fat cells, which have minimal energy requirements, and not at all in red blood cells. Students may wonder how bacteria, which have no mitochondria, obtain metabolic energy. Aerobic bacterial membranes contain enzymes similar to those found in mitochondria, even though they do not have recognizable mitochondrial structures. Mention to the students that one of the enzymes that they used in the laboratory is found in the mitochondria—succinate dehydrogenase. Remind them that the source of this enzyme was a chicken heart.

B-45 THE CHLOROPLAST AND THE SUN

Although the chloroplasts and the mitochondria both have enzymatic pathways involving energy and formation of ATP, the reactions are fundamentally different. The mitochondria break down compounds to form ATP. The chlorophasts use carbon dioxide and light energy from the sun to make ATP and sugars.

A photosynthesis diagram or overlay projection made from the illustration *Conversion of Solar Energy to Metabolic Energy* on page 96 of the student module can be used to illustrate that the ultimate source of energy is the sun. The energy of sunlight is captured and stored in the bonds of carbohydrates. This diagram points out that carbohydrates are merely vehicles for the storage of sunlight energy that has been converted to chemical energy.

1. *Photosynthesis* in plants is an energy-binding *reduction* process.

carbon dioxide + water + energy of sunlight $\xrightarrow{\text{enzymes}}$ sugar + oxygen

$$6CO_2 + 6H_2O + \text{energy of sunlight} \xrightarrow{\text{enzymes}} C_6H_{12}O_6 + 6O_2$$

low potential energy → high potential energy

2. *Respiration* in plants and animals is an energy-releasing *oxidation* process.

sugar + oxygen → carbon dioxide + water + energy of living processes

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy of living processes}$$

high potential energy → low potential energy

Miniexperiment Add a handful of green grass or any other source of chlorophyll (V-8 Juice will work) to an Erlenmeyer flask that contains about 100 cm^3 ethanol. Allow this to sit for about 20 minutes (the alcohol will extract the chlorophyll from the grass). Shine an ultraviolet lamp onto the Erlenmeyer flask and observe the fluorescence caused by the chlorophyll (a ruby-red color). There are many different types of ultraviolet lamps. One that is commercially available is called

Mineralight. This experiment is suggested just for fun. For advanced classes, this could be used as the basis for a discussion of the process of light absorption and fluorescence.

B-46 SEPARATION OF SUBCELLULAR ORGANELLES

This section serves as an introduction to the technique of separating parts of cells by density gradient centrifugation. It gives the student some background on how scientists are able to find out about the parts of the cell. In fractionation, not all of the parts remain whole, but a sufficient number do. When centrifuged, the various constituents separate out in the layers of the density gradient. The particles sink in the density gradient until they reach a level at which the density of the solution is equal to the density of the particles. In a sense, they float on the more dense solution below. The method is an empirical one; the nature of the particles in different layers must be determined.

MINIEXPERIMENT
B-47 SUBCELLULAR FRACTIONATION

This miniexperiment is intended as a model for the methods by which materials can be separated on the basis of density. Although the procedure is not exactly like subcellular fractionation, the principle is the same. The purpose of the experiment, then, is to illustrate the principle used in separating subcellular organelles.

Concepts

- Cells are composed of various subcellular particles called *organelles*.
- These organelles can be separated on the basis of their physical properties—in this case, density.

Objectives

- Prepare a sucrose density gradient.
- Demonstrate proficiency in handling a pipet.
- Determine the order of increasing density in a series of beads.

Estimated Time 30 minutes

Student Grouping Individuals or pairs

Materials

15 25 × 200-mm test tubes
15 10-cm^3 pipets, disposable
150 cm^3 water (top layer solution)
150 cm^3 0.44 M sucrose (15 percent solution)
150 cm^3 1.17 M sucrose (40 percent solution)
15 sets of beads, various densities (see below)

A Cellular Fractionation Simulation Kit, including a class set of density beads, can be purchased from Carolina Biological Supply Company, (Burlington, NC 27215 [Catalog No. 20-1200]). Beads may also be improvised from inexpensive automotive antifreeze testers. In the latter case, you will have to work with the sucrose solutions appropriate for the beads obtained.

Advance Preparation Solutions: The top layer is water; the middle layer is 0.44 M sucrose, prepared by dissolving 150 g sucrose in distilled water and diluting to 1 liter. (The density of this solution at room temperature is approximately 1.056 g/cm^3.) The bottom layer is 1.17 M sucrose, prepared by dissolving 400 g sucrose and diluting to 1 liter. (The density of this solution at room temperature is approximately 1.150 g/cm^3.)

It may help students if each solution is tinted with a different color food dye. In this way, they will be able to see if they have a good gradient in the test tube. The solutions should be labeled by percent sucrose rather than by letters. If sucrose solutions are made up very far in advance, you can freeze them to prevent the growth of bacteria.

Prelab Discussion After discussing the density gradient concept as a model for fractionation, you can give the students some tips on how to prepare the gradient. If pipets are to be used, then the procedure set forth in the student module works well (i.e., start with the lightest solution and build the gradient from below). Without a pipet, the student may be able to layer the solutions, starting with the most dense and then carefully running the next one down the side of the test tube, using a stirring rod to guide the stream. This method requires some skill, but it can be done. Once the gradient is ready, the beads should be released from the surface rather than dropped from above it.

Range of Results The experiment should show students that the beads will float at different heights, according to the density of the medium and the beads.

Postlab Discussion You could go into more detail on the general concept of models or the definition of density or the theory of buoyancy, depending on your own and the students' inclinations. The exercise questions also provide material for discussion.

The text mentions the electron microscope, which is another tool used to unravel the mysteries of the cell. Some students may wish to present a report to the class on the workings of the electron microscope. It is good for students to realize that our knowledge of the cell did not arise out of thin air. It took a great deal of work by a great many people, including those who invented the tools of science.

Answers to questions

1. density
2. density gradient separation

B-48 THE NUCLEUS: INFORMATION STOREHOUSE

This section introduces some terminology that will be used in the remainder of the module, and it also focuses on the part of the cell that will be discussed in the next two sections. Many students may have had a biology course that includes a study of DNA, RNA, ribosomes, etc., so these last few sections should give them a clearer idea of what goes on, chemically, in the nucleus.

B-49 STRUCTURE OF NUCLEIC ACIDS

It is important for students to keep clearly in mind what the terms in this section refer to. It is all too easy to get lost in terminology and lose sight of the important subject matter. The concepts of nucleotide base pairing by hydrogen bonding and nucleotide sequences are critical to understanding how DNA works. Perhaps students will realize what an impact these discoveries had on our understanding of the life process. Again, they should be made aware of the fact that the information which is briefly summarized in the text is the result of a tremendous human effort.

B-50 DOUBLE ROLE OF THE DOUBLE HELIX

The concept of base pairing in the previous section is used to explain the function of DNA as a template for the synthesis of new DNA and RNA. The students should be able to see the similarity in these two processes, which are fundamental to the double role of DNA. Newly synthesized DNA is used to fulfill the first role—that is, cell replication (reproduction). The RNA molecules that are synthesized are used to translate the genetic information into specific proteins. The proteins (mostly enzymes) carry on the processes that characterize each cell type. Thus the second role of DNA is accomplished using RNA as an intermediate.

The material in this section leads directly into the next section, and new terms introduced should be clearly explained so that the student does not become lost in terminology. If it is not obvious to the student, point out that the nucleotide sequence is extremely important in that it contains the coded genetic information. The nature of the code itself is discussed in the next section.

Miniexperiment A useful DNA Model Kit is available from K. D. Biographics (1050 Flake Drive, Palatine, IL 60067). This kit covers RNA and protein synthesis and may also be useful in section *B-51*.

B-51 RIBOSOMES AND PROTEIN SYNTHESIS

We now move out of the nucleus into the other parts of the cell to show how the nuclear processes affect the whole organism. You may have to spend some time with the concepts of language and codes so students will see that the number of combinations for any given word size is a matter of mathematics, not magic. You might reflect on how much effort it took, on the part of some of our finest scientists, to discover how to spell with three-letter words.

Point out that the material in this section completes our description of the biochemical basis of heredity. At this time the students may profit from an example illustrating the general sequence of events involved in the expression of hereditary traits. For example, blue eyes are an inherited trait resulting from the production of certain pigments that are synthesized because the information in the DNA sequences responsible for eye color is passed along through the RNA to the ribosomes.

At the ribosome, the enzymes responsible for the synthesis of the pigments are produced.

A central theme for this section might be the amazingly intricate cooperation required for protein synthesis to occur. The three types of RNA that were synthesized in the nucleus must come together at the ribosome. At the same time the cell must supply the energy (from the mitochondria) to link the amino acids into growing protein chains. Thus the nucleus, mitochondria, ribosomes, and cytoplasm are all involved in this fundamental phenomenon.

ANSWERS TO EXERCISES

(Student module page 111)

1. The functions of these organelles are described in the student module.
2. thymine
3. uracil
4. adenine, because uracil is most similar to thymine, which pairs with adenine in the DNA molecule
5. T-A-G-T-A-C
6. U-A-G-U-C
7. 372
8. No, 2 bases taken three at a time would only give 8 unique combinations and 20 are needed.

EVALUATION ITEMS

These are additional evaluation items that you may wish to use with your students at various times during the preceding section. The correct answer to each question is indicated by shading.

1. Which of the following molecules would diffuse through a dialysis membrane?

   A. fructose C. catalase
   B. glycogen D. hemoglobin

2. On what basis does a dialysis membrane separate biomolecules?

   In dialysis, molecules are separated on the basis of size. Small molecules can pass through the membrane, large ones cannot.

3. In which subcellular organelle are the enzymes for the Krebs cycle found?

   the mitochondrion

4. Which of the following does not occur in the mitochondrion?

   A. Krebs cycle C. ATP formation
   B. respiratory chain D. glycolysis

5. In which of the following does photosynthesis take place?

   A. chloroplast C. cytoplasm
   B. mitochondrion D. nucleus

6. What principle is used in the process of subcellular fractionation?

   Particles are separated according to their densities.

7. All cells contain nuclei.

   T or F (red blood cells and bacteria do not)

8. Explain where DNA is found in animal cells.

   DNA is found in the nucleus of the cell.

9. Given the following part of a DNA strand, C C G T A T C, which would be the corresponding RNA counterpart?

   A. G G C U U U G C. G G C A T A C
   B. G G C U T U G D. G G C A U A G

10. The nucleotide bases pair in a particular way in the DNA molecule because

    A. the length of the pairs is critical.
    B. the hydrophobic bonding is critical.
    C. the length and hydrogen bonding are critical.
    D. the length and hydrophobic bonding are critical.

11. The pairs of bases in DNA are held together by:

    A. ionic bonds C. phosphate groups
    B. hydrogen bonds D. deoxyribose groups

12. Which of the following does not belong in RNA?

    A. thymine D. nucleotide
    B. phosphate E. ribose
    C. uracil

13. The organelle at which protein synthesis takes place is the

    A. nucleus. C. cell membrane.
    B. mitochondrion. D. ribosome.

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14. If there were 66 known amino acids, what would be the smallest word size that could account for the genetic code?

A. two B. three [C.] four D. five

15. The nucleic acid responsible for collecting specific amino acids and depositing them on the ribosomal template is:

A. DNA B. m-RNA C. r-RNA [D.] t-RNA

Where Are We?

We end this module with practical examples of how the students' knowledge of the structure of biomolecules can be used to explain diseases and other day-to-day problems on the basis of simple chemical and physical changes.

The impact of biochemical research and discoveries on our lives may be developed as a major discussion theme through a review of the news articles your students have collected since they began studying biochemistry. Focus on questions along these lines: What are the major problems in biochemistry today? What progress has been made in the short time the students have been collecting these articles? Are there any research breakthroughs in sight? If so, for how long has the topic been under research? During the time your students have been studying *Molecules in Living Systems,* have any new areas of concern to the biochemist become important?

In addition, students may wish to discuss the topics touched on briefly in the summary section: sickle-cell anemia, diabetes, recombinant DNA, genetic disease and mutations, phosphate fertilizers, and ammonia and nitrogen fixation. Assessment of your students' interests and abilities will determine the direction you take in furthering this type of biochemistry discussion. Previous IAC students have found biochemistry relevant, lively, and fascinating; we hope your students are equally enthusiastic.

Appendix

Safety

SAFETY IN THE LABORATORY

Proper conduct in a chemistry laboratory is really an extension of safety procedures normally followed each day around your home and in the outside world. Exercising care in a laboratory demands the same caution you apply to driving a car, riding a motorbike or bicycle, or participating in a sport. Athletes consider safety measures a part of playing the game. For example, football players willingly spend a great deal of time putting on equipment such as helmets, hip pads, and shoulder pads to protect themselves from potential injury.

Chemists must also be properly dressed. To protect themselves in the laboratory, they commonly wear a lab apron or a coat and protective glasses. Throughout this course you will use similar items. Hopefully their use will become second nature to you, much as it becomes second nature for a baseball catcher to put on a chest protector and mask before stepping behind home plate.

As you read through a written experimental procedure, you will notice that specific hazards and precautions are called to your attention. Be prepared to discuss these hazards with your teacher and with your fellow students. Always read the entire experimental procedure thoroughly before starting any laboratory work.

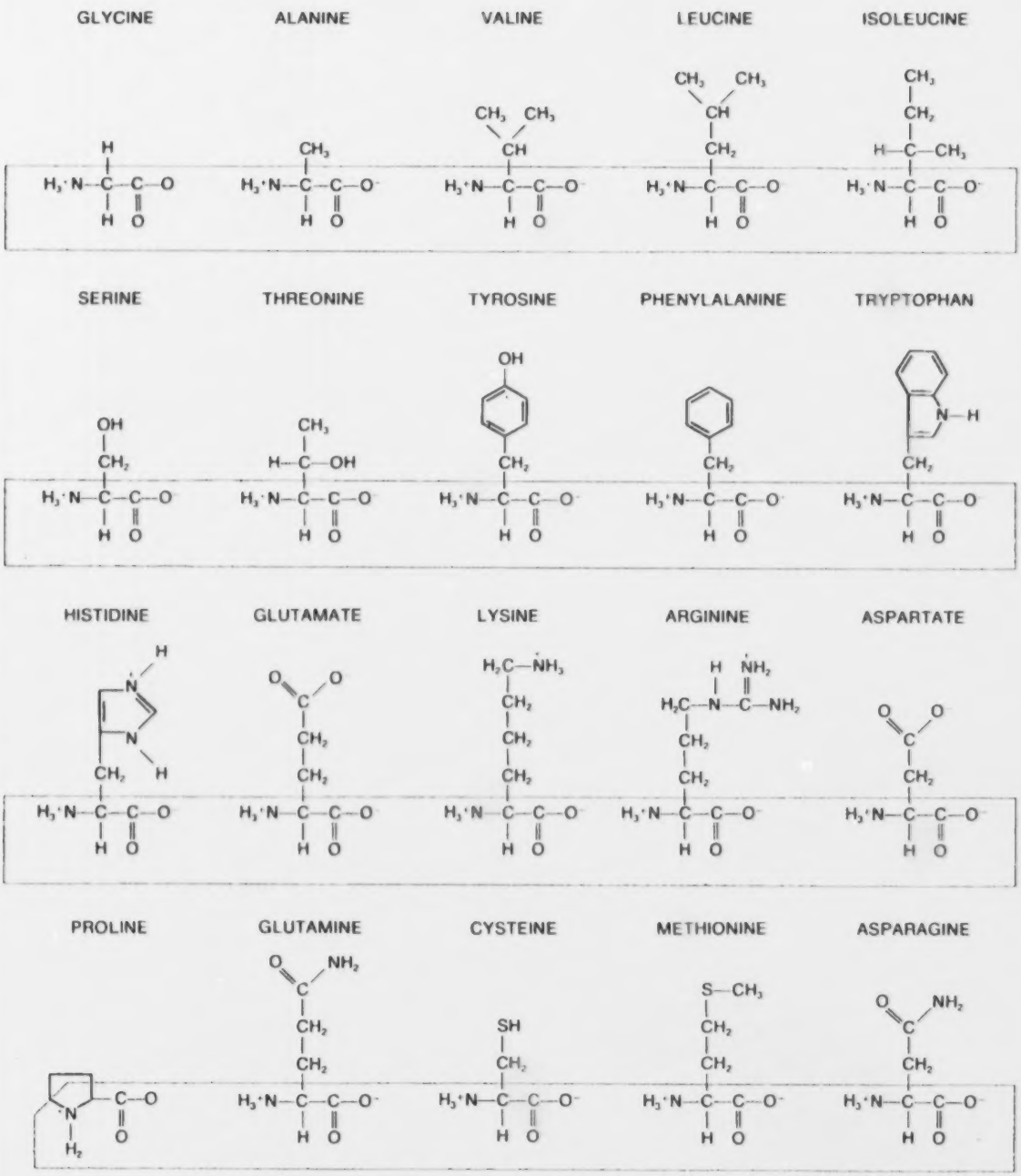
A list of general laboratory safety procedures follows. It is not intended that you memorize these safety procedures but rather that you *use* them regularly when performing experiments. You may notice that this list is by no means complete. Your teacher may wish to add safety guidelines that are relevant to your specific classroom situation. It would be impossible to anticipate every hazardous situation that might arise in the chemistry laboratory. However, if you are familiar with these general laboratory safety procedures and if you use common sense, you will be able to handle potentially hazardous situations intelligently and safely. Treat all chemicals with respect, not fear.

GENERAL SAFETY GUIDELINES

1. Work in the laboratory only when the teacher is present or when you have been given permission to do so. In case of accident, notify your teacher immediately.
2. Before starting any laboratory exercise, be sure that the laboratory bench is clean.
3. Put on a laboratory coat or apron and protective glasses or goggles before beginning an experiment.
4. Tie back loose hair to prevent the possibility of its contacting any Bunsen burner flames.
5. Open sandals or bare feet are not permitted in the laboratory. The dangers of broken glass and corrosive liquid spills are always present in a laboratory.
6. Fire is a special hazard in the laboratory because many chemicals are flammable. Learn how to use the fire blanket, fire extinguisher, and shower (if your laboratory has one).
7. For minor skin burns, immediately immerse the burned area in cold water for several minutes. Then consult your teacher for further instructions on possible additional treatment.
8. In case of a chemical splash on your skin, immediately rinse the area with cold water for at least one minute. Consult your teacher for further action.
9. If any liquid material splashes into your eye, wash the eye immediately with water from an eyewash bottle or eyewash fountain.
10. Never look directly down into a test tube—view the contents of the tube from the side. (Why?)
11. Never smell a material by placing your nose directly at the mouth of the tube or flask. Instead, with your hand, "fan" some of the vapor from the container toward your nose. Inhale cautiously.
12. Never taste any material in the laboratory.
13. Never add water to concentrated acid solutions. The heat generated may cause spattering. Instead, as you stir, add the acid slowly to the water or dilute solution.
14. Read the label on a chemical bottle at least *twice* before removing a sample. H_2O_2 is not the same as H_2O.
15. Follow your teacher's instructions or laboratory procedure when disposing of used chemicals.

This symbol represents three of the common hazards in a chemistry laboratory—flame, fumes, and explosion. It will appear with certain experiments in this module to alert you to special precautions in addition to those discussed in this Appendix.

20 Common Amino Acids



The 20 amino acids illustrated here are commonly found in proteins. Each has a different R group or side chain, attached to the central carbon atom, so that the various amino acids exhibit a wide range of chemical properties. The amino acids are shown in the ionic forms present in biological systems.

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Metric Units

PHYSICAL QUANTITY	SI BASE OR DERIVED UNIT		OTHER UNITS	
	NAME	SYMBOL AND DEFINITION	NAME	SYMBOL AND DEFINITION
length	meter*	m	kilometer centimeter nanometer	1 km = 10^3 m 1 cm = 10^{-2} m 1 nm = 10^{-9} m = 10^{-7} cm
area	square meter	m^2	square centimeter	1 cm^2 = 10^{-4} m^2
volume	cubic meter	m^3	cubic centimeter liter	1 cm^3 = 10^{-6} m^3 1 l = 10^3 cm^3
mass	kilogram*	kg	gram	1 g = 10^{-3} kg
time	second*	s		
amount of substance	mole*	mol	millimole micromole nanomole	1 m mol = 10^{-3} mol 1 μ mol = 10^{-6} mol 1 n mol = 10^{-9} mol
concentration	moles per cubic meter	mol/m^3	moles per liter molar concentration (molarity)	1 mol/l = 10^3 mol/m^3 1 M = mol/l
Celsius temperature			degree Celsius	°C

*SI base unit, exactly defined in terms of certain physical measurements.

Selected Readings and Films

ARTICLES

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FILMS

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Biochemistry and Molecular Structure. Color, 22 minutes.

Catalysis, Color, 17 minutes.

Module Tests

Two module tests follow, one to test knowledge-centered objectives and the other to test skill-centered objectives. If you choose to use either or both of these module tests as they are presented here, duplicate copies for your students. Or, you may wish to select some of the questions from these tests that you feel apply to *your* introductory chemistry course and to add questions of your own. Either way, make sure the test that you give reflects your emphasis on the chemistry that you and your students experienced in this introductory biochemistry module. The skill-centered tests will require that you set up several laboratory stations containing materials for your students to examine or work with. You may wish to incorporate additional test items to round out the types of skills you and your students have worked on. (Answers to the test questions in this section are provided.) If you wish to use a standard-type answer sheet for this test, one is provided on page 67 of this guide. Duplicate in quantities sufficient for your classroom use.

ANSWER KEY FOR THE KNOWLEDGE-CENTERED MODULE TEST

1. D; **2.** C; **3.** C; **4.** C; **5.** D; **6a.** C; **6b.** A; **6c.** D; **7.** C; **8.** A; **9.** B; **10.** C; **11.** B; **12.** D; **13.** B; **14.** A; **15.** A; **16.** D; **17.** C; **18.** B; **19.** B; **20a.** A; **20b.** D; **20c.** B; **21.** D; **22.** C; **23.** A; **24.** D; **25.** B; **26.** B; **27.** A

MOLECULES IN LIVING SYSTEMS
Knowledge-Centered Module Test

1. Subcellular organelles can be separated by the use of:

   A. dialysis
   B. titration
   C. hydrophobic solvents
   D. density gradients

2. A student was given four biomolecules—1, 2, 3, and 4. Each compound was placed in dialysis tubing, and the appropriate tests showed that only compound 1 migrated through the dialysis tubing. the statement that best supports this observation is:

   A. Compound 1 is a macromolecule.
   B. Compounds 2, 3, and 4 are amino acids.
   C. Compounds 2, 3, and 4 are macromolecules.
   D. Compounds 2, 3, and 4 are simple molecules.

3. An enzyme is most effective in catalyzing a reaction at a pH of 7.2. If the pH is raised to 11, one would predict that

   A. the reaction rate will be unchanged.
   B. the reaction rate will be greater than before.
   C. the reaction rate will be less than before.
   D. Insufficient information is given to answer the question.

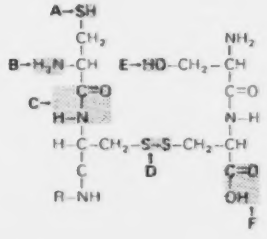
4. Every step in a metabolic pathway involves:

   A. NAD B. ATP C. enzymes D. all of these

5. A student is given an unknown compound and asked to run four tests on the compound. Ninhydrin and Biuret give a positive test, while iodine and Benedict's give negative tests. The compound is

   A. a simple sugar.
   B. a polysaccharide.
   C. an amino acid.
   D. a protein.

6. Refer to the following biomolecule in answering questions a, b, and c. Shaded areas that are lettered indicate functional groups. Use these letters to determine your answers.



   a. A peptide link is represented by:

      A. A B. B C. C D. D

   b. A thiol group is represented by:

      A. A B. B C. D D. F

   c. The carboxylic acid group is represented by:

      A. B B. D C. E D. F

7. Small changes in pH can cause large changes in the folding of a protein because

   A. covalent bonds are broken.
   B. disulfide bridges are broken.
   C. ionic bonds are broken.
   D. all of the above

8. In an enzyme experiment, a student added 2 cm^3 of blue 0.0004 M DPIP to a reaction mixture of succinate and succinate dehydrogenase. The blue color disappeared in 4.30 minutes. If the reaction ratio is one DPIP to one succinate, the reaction rate is

   A. 1.86×10^{-7} moles/minute.
   B. 9.30×10^{-7} moles/minute.
   C. 3.72×10^{-5} moles/minute.
   D. 4.67×10^{-4} moles/minute.

9. The main function of the cell's nucleus is to

A. supply the enzymes used in the Krebs cycle.
B. synthesize RNA and DNA for the cell.
C. supply the enzymes used in glycolysis.
D. digest lipids.

10. The reaction in which an amino acid is acting as a base is:

A. $H_2H—CH_2—COOH + NH_3 \longrightarrow H_2N—CH_2—COO^- + NH_4^+$

B. $^+H_3N—CH_2—COO^- + KOH \longrightarrow H_2N—CH_2—COO^- + K^+ + H_2O$

C. $^+H_3N—CH_2—COO^- + HCl \longrightarrow {}^+H_3N—CH_2—COOH + Cl^-$

D. all of the above

11. Some molds and bacteria produce acetic acid and lactic acid in the metabolism of glucose. This process is called the

A. hydrolysis of carbohydrates.
B. fermentation of carbohydrates.
C. denaturing of carbohydrates.
D. dialysis of carbohydrates.

12. The kidney machine is a device that functions in a manner similar to

A. a density gradient.
B. Benedict's solution.
C. an enzyme.
D. dialysis tubing.

13. In the following reaction

$$^+H_3N—CH_2—CH_2—COO^- + ROH \xrightarrow[\mathbf{X}]{\text{enzyme}} {}^+H_3N—CH_2—CH_2—COOR + OH^-$$

$^+H_3N—CH_2—CH_2—COO^-$ is a substrate for enzyme **X**. Another suitable substrate would be:

A. $^+H_3N—CH_2—CH_2—CH_3$
B. $^+H_3N—CH_2—COO^-$
C. $^-OOC—CH_2—CH_2—COO^-$
D. $CH_3—CH_2—CH_2—COO^-$

14. Proteins and enzymes are classified with amino acids because

A. proteins and enzymes are composed of amino acids.
B. proteins and enzymes are smaller than amino acids.
C. they are all lipids.
D. they all catalyze reactions that occur in the body.

15. The molecule that carries genetic information from the nucleus to the ribosomes and serves as the template for protein synthesis is

A. messenger RNA.
B. ribosomal RNA.
C. transfer RNA.
D. DNA.

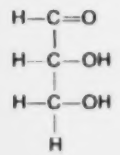
16. A student is given four sugar solutions

A—15% sugar
B—13% sugar
C—9% sugar
D—11% sugar

When preparing a density gradient in a large test tube by means of the pipet method, the student should place the solutions into the test tube in the following order:

A. DCBA B. ABCD C. CBDA D. CDBA

17. The following biomolecule



is an example of a

A. protein.
B. nucleic acid.
C. carbohydrate.
D. lipid.

18. The solvent(s) likely to dissolve this biomolecule is (are):

A. C_8H_{18}
B. H_2O
C. C_6H_{14}
D. all of the above

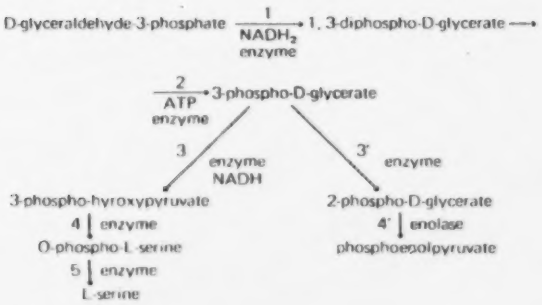
19. In the reaction

$2\ H_2O_2 + \text{catalase} \longrightarrow \text{catalase} + 2\ H_2O + O_2$

H_2O_2 is

A. an enzyme.
B. a reactant.
C. an inorganic catalyst.
D. a cofactor.

20. Examine the following metabolic pathway and use the information to answer questions a., b., and c.



a. A metabolite is represented by:

A. L-serine
B. enolase
C. ATP
D. all of the above

b. A branch point is shown at:

A. 1,3-diphospho-D-glycerate
B. 2-phospho-D-glycerate
C. D-glyceraldehyde-3-phosphate
D. 3-phospho-D-glycerate

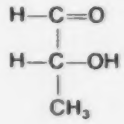
c. A cofactor is:

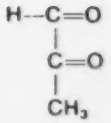
A. L-serine
B. ATP
C. O-phospho-L-serine
D. 3-phospho-D-glycerate

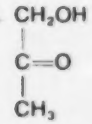
21. Four bases that are contained in the DNA molecule are adenine, cytosine, guanine, and thymine. The base pairing that occurs in the DNA molecule is

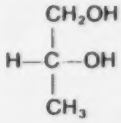
A. C—T only.
B. C—T and A—G.
C. A—C and G—T.
D. A—T and G—C.

22. Choose an isomer(s) of the following molecular structure from the answers below.



A. 

C. 

B. 

D. all of the above

23. If a protein is heated, the hydrogen bonds are broken and the protein is

A. denatured.
B. hydrolyzed.
C. metabolized.
D. fermented.

24. The organelle mainly responsible for metabolic energy production is the

A. cell membrane.
B. ribosome.
C. nucleus.
D. mitochondrion.

25. The change of one base in the DNA molecule can result in sickle-cell anemia because

A. the Krebs cycle enzymes are never produced.
B. the change of the base results in the incorrect sequence of amino acids in newly produced protein.
C. the hemoglobin formed does not contain enough iron to carry out necessary body functions.
D. no hemoglobin is formed.

26. The DNA molecule contains four different bases —A, G, C, and T. These bases are grouped into triplets that act as words containing three letters each, such as ACT. This is the smallest word size that can account for the genetic code because

A. this gives 16 three-letter words to account for only 16 amino acids.
B. this gives 64 three-letter words to account for only 20 amino acids.
C. this gives 256 three-letter words to account for only 20 amino acids.
D. none of these

27. Three compounds, A, B, and C, are added to an enzyme as shown below and their reaction rates are measured.

Tube	*Buffer* cm^3	*Enzyme* cm^3	*A* cm^3	*B* cm^3	*C* cm^3	H_2O cm^3	*Reaction Rate*
1	8	2	1	0	0	1	300
2	8	2	0	1	0	1	0
3	8	2	0	0	1	1	0
4	8	2	1	1	0	0	0
5	8	2	1	0	1	0	0
6	8	2	0	0	0	2	0

Select the statement that best illustrates the above data:

A. Substances B and C are acting as enzyme inhibitors.

B. Substance A is an enzyme.

C. Tube #3 shows that C is a suitable substrate for the enzyme.

D. Tube #1 is a blank, or control.

Skill-Centered Module Test

If you decide to use these skill-centered test items, you will need to make certain advance preparations. The numerals in the following list indicate the items for which you will have to prepare special laboratory stations. Be sure to test each of the lab stations before allowing students to determine the answers to the skill-centered items. When students are ready to answer these questions, they should go to the numbered station and follow the directions that are given there and in the printed question item. When they finish with the materials at the station, instruct them to leave the materials in proper order for the next student.

1 Provide the following sugars on watch glasses:

Sugar A—fructose Sugar B—lactose
Sugar C—galactose Sugar D—maltose

2 Provide 4 test tubes numbered 1, 2, 3 and 4. Label the 5-cm^3 level with a ring. Provide a graduated cylinder and a corked flask of hexane or other aliphatic hydrocarbon.

Provide Solid A (KNO_3 or NaCl) on a watch glass along with a spatula.

Provide Solid B (naphthalene) on a different watch glass along with a spatula.

4 Set up 4 test tubes labeled 1, 2, 3, and 4. Put a piece of tape or crayon mark at the 3-cm^3 mark on each test tube. Provide a beaker of 3 percent H_2O_2. Mark beaker: **Caution.**

Put Solid A (NaCl) on a watch glass along with a spatula.
Put Solid B (sugar) on a watch glass along with a spatula.
Put Solid C (MnO_2) on a watch glass along with a spatula.
Put Liquid D (water) in a beaker with a medicine dropper.

7 Make the "sauerkraut juice" as 1.0 M HCl. Dilute 20 cm^3 of concentrated HCl to a final volume of 250 cm^3. Place the solution in a buret with extra in a bottle. Have a funnel handy to refill the buret. Provide a dropper bottle of phenolphthalein.

Fill a buret with 1 M NaOH (10 g/250 cm^3 H_2O).

Provide extra in a bottle labeled *base*. Have a funnel marked *base* handy.

You will have to titrate the sample yourself to know the correct answer.

10 Provide pH paper in the range 10–14. Dissolve 0.4 g NaOH in 1 liter H_2O for the test solution. Provide a stirring ro[illegible]

ANSWER KEY FOR THE SKILL-CENTERED MODULE TEST

1. A/C; **2.** B; **3a.** D; **3b.** B; **4.** C; **5.** C; **6.** A; **7.** *; **8.** A; **9.** C; **10.** *; **11.** B; **12.** D

*Evaluate according to teacher standards

MOLECULES IN LIVING SYSTEMS
Skill-Centered Module Test

Several questions in this section require you to make observations and perform chemical manipulations. The stations where you will do these operations will be indicated by your teacher. If the station you are going to is being used, continue with the test and go back later.

1. Go to station #1 and examine the four sugars provided. By conducting simple taste tests, determine which sugar is the sweetest. The sweetest sugar is:

   A. A B. B C. C D. D

2. Go to station #2, where you will find four test tubes numbered 1, 2, 3, and 4. The 5-cm^3 level is indicated by a ring. Add water to tubes 1 and 2 up to the mark and add hexane to tubes 3 and 4 up to the mark. Add a pinch of solid A to tubes 1 and 3 and a pinch of B to tubes 2 and 4. The nonpolar materials(s) is (are):

   A. A B. B C. A and B D. neither A nor B

   Rinse out all test tubes before leaving.

3. Examine the following table and determine the answers to questions a and b.

Substance Tested	*Test Performed*			
	Iodine	*Biuret*	*Ninhydrin*	*Benedict's*
A	–	–	–	+
B	–	–	+	–
C	–	+	+	–
D	+	–	–	–

The minus sign ("–") indicates a negative test. The plus sign ("+") indicates a positive test.

   a. The substance on the table that is a starch is:

   A. A B. B C. C D. D

   b. Which substance on the table is a protein?

   A. A B. B C. C D. D

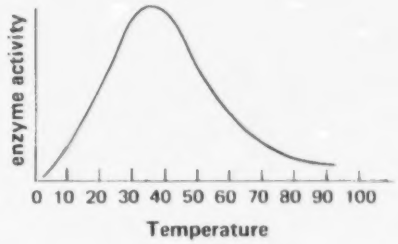
4. Go station #4, where you will find four test tubes in a rack labeled 1, 2, 3, and 4. The 3-cm^3 mark is indicated by a ring. Pour 3 cm^3 of peroxide into each tube. To tube #1 add a few grains of A, to tube #2 add a few grains of B. To tube #3 add a few grains of C, and to tube #4 add a few drops of D. The substance that acted as a catalyst is:

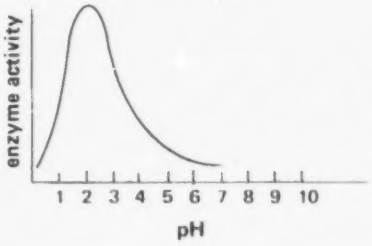
   A. A B. B C. C D. D

5. The evidence for making your decision in question 5 was a(n)

   A. evolution of heat. C. evolution of gas.
   B. change of color. D. formation of precipitate.

6. Examine the graphs of enzyme activity as influenced by pH and temperature for the stomach enzyme pepsin.





   From the above graphs you would conclude that the enzyme has maximum activity at

   A. pH 2 and temperature of 35°C.
   B. pH 2 and temperature of 50°C.
   C. pH 3 and temperature of 50°C.
   D. pH 3 and temperature of 35°C.

7. Go to station #7 and, using the laboratory apparatus provided, titrate a 10-cm^3 sample of commercial sauerkraut juice. Use the standard base provided and phenolphthalein as your indicator. Record the volume of base needed to neutralize the sauerkraut juice on your answer sheet next to #7.

8. A student titrated a 50-cm^3 sample of two-week-old sauerkraut juice. The titration required 25 cm^3 of 0.10 M sodium hydroxide. The number of moles of acid in the juice is:

   A. 0.0025 B. 0.010 C. 0.020 D. 0.0050

9. Which test would you conduct to help prove that a biochemical sample was a sugar?

   A. Biuret C. Benedict's
   B. ninhydrin D. iodine

10. Go to station #10 and, using the pH paper provided, measure the pH of the solution in the Erlenmeyer flask. Record the pH next to #10 on your answer sheet.

11. Examine the data given below:
    The catalyst with the largest influence on rate of reaction is:

    A. I C. I and II are equal
    B. II D. cannot tell from data provided

12. Using the data from question 11, when the concentration of X = 2, Y = 1, Catalyst I = 1, and Catalyst II = 2, you would predict the rate of reaction to be:

    A. 4.0 moles/hour C. 3.0 moles/hour
    B. 6.0 moles/hour D. 8.0 moles/hour

Concentration X (moles/liter)	*Concentration Y (moles/liter)*	*Concentration Catalyst I (moles/liter)*	*Concentration Catalyst II (moles/liter)*	*Rate of Reaction (moles/hour)*
1	1	1	1	1.0
2	1	1	1	2.0
2	2	1	1	4.0
1	1	2	1	1.0
1	1	1	2	4.0

IAC TEST ANSWER SHEET

NAME		SCORE
DATE	CLASS PERIOD	
TEACHER		

Test Type (check)

☐ KNOWLEDGE-CENTERED
☐ SKILL-CENTERED

Module Test (check)

☐ INTRODUCTORY ☐ ORGANIC ☐ INORGANIC ☐ NUCLEAR
☐ PHYSICAL ☐ ENVIRONMENTAL ☐ BIOCHEMICAL ☐ COMPREHENSIVE

	A	B	C	D		A	B	C	D		A	B	C	D		A	B	C	D
1.	☐	☐	☐	☐	11.	☐	☐	☐	☐	21.	☐	☐	☐	☐	31.	☐	☐	☐	☐
2.	☐	☐	☐	☐	12.	☐	☐	☐	☐	22.	☐	☐	☐	☐	32.	☐	☐	☐	☐
3.	☐	☐	☐	☐	13.	☐	☐	☐	☐	23.	☐	☐	☐	☐	33.	☐	☐	☐	☐
4.	☐	☐	☐	☐	14.	☐	☐	☐	☐	24.	☐	☐	☐	☐	34.	☐	☐	☐	☐
5.	☐	☐	☐	☐	15.	☐	☐	☐	☐	25.	☐	☐	☐	☐	35.	☐	☐	☐	☐
6.	☐	☐	☐	☐	16.	☐	☐	☐	☐	26.	☐	☐	☐	☐	36.	☐	☐	☐	☐
7.	☐	☐	☐	☐	17.	☐	☐	☐	☐	27.	☐	☐	☐	☐	37.	☐	☐	☐	☐
8.	☐	☐	☐	☐	18.	☐	☐	☐	☐	28.	☐	☐	☐	☐	38.	☐	☐	☐	☐
9.	☐	☐	☐	☐	19.	☐	☐	☐	☐	29.	☐	☐	☐	☐	39.	☐	☐	☐	☐
10.	☐	☐	☐	☐	20.	☐	☐	☐	☐	30.	☐	☐	☐	☐	40.	☐	☐	☐	☐

For Skill-Centered Tests only, enter the numbers of all special questions and your answers in the spaces below.

Materials List

Quantities listed are for a class of 30 students working in pairs.

*Optional Items. These items depend on teacher choice. We have listed substitutions in the experiment discussion. Consult the specific experiment in the teacher's guide to determine use and quantities.

NONEXPENDABLE MATERIALS

Item	*Experiment*	*Amount*
Balances, 0.01 g sensitivity	—	—
Beakers, 50-cm³	13, 16, 20, 29, 31	30
Beakers, 150-cm³	13, 20, 29, 31, 39, 42	30
Beakers, 250-cm³	29	30
Blender	25, 27	1
Bunsen burners	13, 16, 20, 25, 42	15
Burets, 50-cm³	13*, 23*, 39	15
Burets, 50-cm³, Teflon stopcock	10*	1–3*
Buret clamps, double	13*, 23*, 39	15
Centrifuge	25*, 27*	1*
Clamps, universal	13	15
Clock, for timing	25, 27	1
Dropper bottles, 50-cm³	10, 13, 16, 23, 27, 34	30
Erlenmeyer flasks, 250-cm³	39	15
Funnels, 75-mm diameter	42	15
Funnel supports	42	15
Graduated cylinders, 10-cm³	10, 29, 31, 42	30
Grater, cabbage	39	2–5
Jars, 120-cm³, wide-mouth with lids	39	15
Medicine droppers	13, 16, 20, 23, 34	15
Model kits, DNA	49*, 50*, 51*	15*
Model kits, molecular	3*, 4*, 5*, 6*, 27*	15*
Mortars and pestles	34	15
Petri dishes	20	15
Plastic-bead hydrometers	47	15
Ring stands and rings	13, 16, 20, 23, 29, 34, 42	15
Spatulas	10, 16, 34	15
Stirring rods	31, 39	30
Test tubes, 18 × 150-mm	13, 16, 23, 25, 27, 29, 34, 42	120
Test tubes, 25 × 200-mm	10, 47	60

NONEXPENDABLE MATERIALS (cont.)

Item	*Experiment*	*Amount*
Test-tube clamps	13, 42	15
Test-tube racks	10, 13, 16, 23, 25, 27, 34, 42, 47	15
Thermometers, −10°C to 110°C	16, 20, 29	15
Triangles, clay	42	15
Wire gauze, asbestos centers	13, 16, 20, 42	15

EXPENDABLE MATERIALS

Item	*Experiment*	*Amount*
Adenosine triphosphate (ATP)	34	50 mg
Cabbages, fresh	39	3
†Copper sulfate, pentahydrate	13, 42	25 g
Dialysis tubing, 1.6-cm diameter	42	180 cm
2,6-Dichlorophenolindophenol sodium salt (DPIP)	25, 27	0.1 g
Eggs, fresh	20	2
Fireflies (lanterns)	34	150 approx.
Fructose	6	30 g
Galactose	6	30 g
Gelatin	10, 13, 31, 42	30 g
Glucose (dextrose)	6, 10, 13, 42	50 g
Graph paper, linear	25, 29	60 sheets
Hearts, fresh chicken	25	3–5
Hexane (or TTE)	10	750 cm^3
Hydrochloric acid, conc.	20, 23	20 cm^3
Hydrogen peroxide, 3 percent	16	100 cm^3
Iodine, solid	13, 42	4 g
Lactose	6	30 g
Liver juice *or* blood	16	15 cm^3
Magnesium sulfate, heptahydrate	34	1 g
Malonic acid	27	2.1 g
Maltose	6	30 g
Manganese dioxide	16	5 g
Meat tenderizer	31	25 g
Milk, skim	29	2 liters
Monosodium glutamate (MSG)	10, 13, 42	20 g
Ninhydrin	13, 42	1.5 g
pH paper, universal, wide range	23, 39	50 strips
Phenolphthalein	39	small bottle
Pipet, 1-cm^3, disposable	25, 27, 29	45
Pipet, 10-cm^3, disposable	47	15
Potassium iodide	13, 42	10 g
Propionic acid	27	1.5 g
Pyridine	13, 42	20 cm^3

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EXPENDABLE MATERIALS (cont.)

Item	*Experiment*	*Amount*
Rennet tablets	29	15
Sand (SiO_2)	34	15 g
†Sodium carbonate, anhydrous	13, 42	100 g
Sodium chloride	16, 39	50 g
†Sodium citrate	13, 42	180 g
Sodium hydroxide	13, 39, 42	65 g
Sodium phosphate, dibasic, heptahydrate, $Na_2HPO_4 \cdot 7H_2O$	25	60 g
Sodium phosphate, monobasic, monohydrate, $NaH_2PO_4 \cdot H_2O$	25	30 g
†Sodium potassium tartrate	13, 42	12 g
Sodium succinate, hexahydrate	25, 27	6 g
Spoons, plastic	6	30
Starch, soluble	13, 23, 42	3 g
Sucrose (sugar)	6, 47	175 g
Vegetable oil (or mineral oil)	10, 25, 27	300 cm^3

†These are used in the preparation of Biuret and Benedict's reagents. Commercially prepared solutions may be ordered instead (400 cm^3 of each).

Acknowledgments

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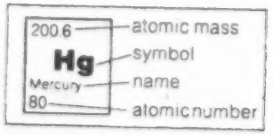
Table of International Relative Atomic Masses*

Element	Symbol	Atomic Number	Atomic Mass
Actinium	Ac	89	227.0
Aluminum	Al	13	27.0
Americium	Am	95	(243)**
Antimony	Sb	51	121.8
Argon	Ar	18	39.9
Arsenic	As	33	74.9
Astatine	At	85	(210)
Barium	Ba	56	137.3
Berkelium	Bk	97	(247)
Beryllium	Be	4	9.01
Bismuth	Bi	83	209.0
Boron	B	5	10.8
Bromine	Br	35	79.9
Cadmium	Cd	48	112.4
Calcium	Ca	20	40.1
Californium	Cf	98	(251)
Carbon	C	6	12.0
Cerium	Ce	58	140.1
Cesium	Cs	55	132.9
Chlorine	Cl	17	35.5
Chromium	Cr	24	52.0
Cobalt	Co	27	58.9
Copper	Cu	29	63.5
Curium	Cm	96	(247)
Dysprosium	Dy	66	162.5
Einsteinium	Es	99	(254)
Erbium	Er	68	167.3
Europium	Eu	63	152.0
Fermium	Fm	100	(257)
Fluorine	F	9	19.0
Francium	Fr	87	(223)
Gadolinium	Gd	64	157.3
Gallium	Ga	31	69.7
Germanium	Ge	32	72.6
Gold	Au	79	197.0
Hafnium	Hf	72	178.5
Helium	He	2	4.00
Holmium	Ho	67	164.9
Hydrogen	H	1	1.008
Indium	In	49	114.8
Iodine	I	53	126.9
Iridium	Ir	77	192.2
Iron	Fe	26	55.8
Krypton	Kr	36	83.8
Lanthanum	La	57	138.9
Lawrencium	Lr	103	(260)
Lead	Pb	82	207.2
Lithium	Li	3	6.94
Lutetium	Lu	71	175.0
Magnesium	Mg	12	24.3
Manganese	Mn	25	54.9
Mendelevium	Md	101	(258)
Mercury	Hg	80	200.6
Molybdenum	Mo	42	95.9
Neodymium	Nd	60	144.2
Neon	Ne	10	20.2
Neptunium	Np	93	237.0
Nickel	Ni	28	58.7
Niobium	Nb	41	92.9
Nitrogen	N	7	14.0
Nobelium	No	102	(259)
Osmium	Os	76	190.2
Oxygen	O	8	16.0
Palladium	Pd	46	106.4
Phosphorus	P	15	31.0
Platinum	Pt	78	195.1
Plutonium	Pu	94	(244)
Polonium	Po	84	(209)
Potassium	K	19	39.1
Praseodymium	Pr	59	140.9
Promethium	Pm	61	(145)
Protactinium	Pa	91	231.0
Radium	Ra	88	226.0
Radon	Rn	86	(222)
Rhenium	Re	75	186.2
Rhodium	Rh	45	102.9
Rubidium	Rb	37	85.5
Ruthenium	Ru	44	101.1
Samarium	Sm	62	150.4
Scandium	Sc	21	45.0
Selenium	Se	34	79.0
Silicon	Si	14	28.1
Silver	Ag	47	107.9
Sodium	Na	11	23.0
Strontium	Sr	38	87.6
Sulfur	S	16	32.1
Tantalum	Ta	73	180.9
Technetium	Tc	43	(97)
Tellurium	Te	52	127.6
Terbium	Tb	65	158.9
Thallium	Tl	81	204.4
Thorium	Th	90	232.0
Thulium	Tm	69	168.9
Tin	Sn	50	118.7
Titanium	Ti	22	47.9
Tungsten	W	74	183.8
Uranium	U	92	238.0
Vanadium	V	23	50.9
Xenon	Xe	54	131.3
Ytterbium	Yb	70	173.0
Yttrium	Y	39	88.9
Zinc	Zn	30	65.4
Zirconium	Zr	40	91.2

*Based on International Union of Pure and Applied Chemistry (IUPAC) values (1975).

**Numbers in parentheses give the mass numbers of the most stable isotopes.

PERIODIC TABLE OF THE ELEMENTS



IA	IIA	IIIB	IVB	VB	VIB	VIIB	← VIIIB →	IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA
															4.00 He Helium 2
6.94 Li Lithium 3	9.01 Be Beryllium 4									10.8 B Boron 5	12.0 C Carbon 6	14.0 N Nitrogen 7	16.0 O Oxygen 8	19.0 F Fluorine 9	20.2 Ne Neon 10
23.0 Na Sodium 11	24.3 Mg Magnesium 12									27.0 Al Aluminum 13	28.1 Si Silicon 14	31.0 P Phosphorus 15	32.1 S Sulfur 16	35.5 Cl Chlorine 17	39.9 Ar Argon 18
39.1 K Potassium 19	40.1 Ca Calcium 20	45.0 Sc Scandium 21	47.9 Ti Titanium 22	50.9 V Vanadium 23	52.0 Cr Chromium 24	54.9 Mn Manganese 25	55.8 Fe Iron 26	58.9 Co Cobalt 27	58.7 Ni Nickel 28	63.5 Cu Copper 29	65.4 Zn Zinc 30	69.7 Ga Gallium 31	72.6 Ge Germanium 32	74.9 As Arsenic 33	79.0 Se Selenium 34	79.9 Br Bromine 35	83.8 Kr Krypton 36
85.5 Rb Rubidium 37	87.6 Sr Strontium 38	88.9 Y Yttrium 39	91.2 Zr Zirconium 40	92.9 Nb Niobium 41	95.9 Mo Molybd'm 42	(97) Tc Technetium 43	101.1 Ru Ruthenium 44	102.9 Rh Rhodium 45	106.4 Pd Palladium 46	107.9 Ag Silver 47	112.4 Cd Cadmium 48	114.8 In Indium 49	118.7 Sn Tin 50	121.8 Sb Antimony 51	127.6 Te Tellurium 52	126.9 I Iodine 53	131.3 Xe Xenon 54
132.9 Cs Cesium 55	137.3 Ba Barium 56	138.9 La* Lanthanum 57	178.5 Hf Hafnium 72	180.9 Ta Tantalum 73	183.8 W Tungsten 74	186.2 Re Rhenium 75	190.2 Os Osmium 76	192.2 Ir Iridium 77	195.1 Pt Platinum 78	197.0 Au Gold 79	200.6 Hg Mercury 80	204.4 Tl Thallium 81	207.2 Pb Lead 82	209.0 Bi Bismuth 83	(209) Po Polonium 84	(210) At Astatine 85	(222) Rn Radon 86
(223) Fr† Francium 87	226.0 Ra Radium 88	(227) Ac** Actinium 89	‡ 104	‡ 105	‡ 106												

*	140.1 Ce Cerium 58	140.9 Pr Praseody'm 59	144.2 Nd Neodymium 60	(145) Pm Promethium 61	150.4 Sm Samarium 62	152.0 Eu Europium 63	157.3 Gd Gadolinium 64	158.9 Tb Terbium 65	162.5 Dy Dysprosium 66	164.9 Ho Holmium 67	167.3 Er Erbium 68	168.9 Tm Thulium 69	173.0 Yb Ytterbium 70	175.0 Lu Lutetium 71
**	232.0 Th Thorium 90	231.0 Pa Protactinium 91	238.0 U Uranium 92	237.0 Np Neptunium 93	(242) Pu Plutonium 94	(243) Am Americium 95	(245) Cm Curium 96	(245) Bk Berkelium 97	(251) Cf Californium 98	(254) Es Einsteinium 99	(254) Fm Fermium 100	(256) Md Mendel'm 101	(254) No Nobelium 102	(257) Lr Lawrencium 103

†The most stable known isotopes are shown in parentheses.

‡The discovery of elements 104, 105, and 106 has been claimed by both American and Soviet scientists. The Americans have suggested the name *rutherfordium* and *hahnium* for 104 and 105; the Soviets have suggested the names *kurchatovium* and *nielsbohrium* for these same elements. No name has yet been proposed for element 106.

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